THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE MONTPELLIER SUPAGRO

En Ecologie Fonctionnelle

École doctorale GAIA – Biodiversité, Agriculture, Alimentation, Environnement, Terre, Eau

Unité de recherche UMR Eco&Sols

En partenariat international avec
Universidade Estadual Paulista « Júlio de Mesquita Filho », Brésil

Consequences of clear-cutting on the production of fine roots, CO_2, CH_4 and N_2O down to the water table in *Eucalyptus grandis* stands conducted in coppice in a throughfall-exclusion experiment.

Présentée par Amandine GERMON
Le 21 Juin 2019

Sous la direction de Jean-Paul LACLAU
et Christophe JOURDAN

Devant le jury composé de

Alain PIERRET, Chargé de Recherche, IRD	Rapporteur
Daniel EPRON, Professeur, Université de Lorraine	Rapporteur
Jean-Christophe DOMEC Professeur, Bordeaux Sciences Agro	Examinateur
Catherine ROUMET, Chargé de Recherche, CEFE/CNRS, Montpellier	Invitée
José Leonardo Gonçalves, Professeur, ESALQ, USP Brésil	Invité
Jean-Paul LACLAU, Professeur, UNESP Brésil	Directeur de thèse
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UNIVERSITÉ DE MONTPELLIER

Montpellier SupAgro

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24048 images prises dans les minirhizotrons et analysées ;
3828 seringues collectés et analysés soit 684 heures passées au « labo de la mort » ;
2022 échantillons de sols collectés et 1820 analyses de N minéral au laboratoire ;
307 m de sol collecté soit 578 échantillons de sols lavés pour séparer les racines ;
Plus de 400 descentes dans les fosses soit bien plus de 400 heures passées sous terre ;
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ABSTRACT

Tree growth is highly dependent on the absorptive function of fine roots for water and nutrients. Fine roots also play a major role in the global carbon (C) cycle, mainly through production, respiration, exudation and decomposition processes. Improving our understanding of the spatiotemporal dynamics of fine roots and greenhouse gases in deep soil layers is a key component to identify more sustainable silvicultural practices for planted forests in a context of climate change and to improve the current biogeochemical models.

Our study aimed to assess the effect of clear-cutting and drought on fine-root production, soil CO_2, CH_4 and N_2O effluxes and production throughout deep soil profiles down to the water table in Brazilian coppice-managed *Eucalyptus* plantations. Fine roots (i.e. diameter < 2 mm) were sampled down to a depth of 17 m in a throughfall exclusion experiment comparing stands with 37% of throughfall excluded by plastic sheets (-W) and stands without rain exclusion (+W). Root dynamics were studied using minirhizotron in two permanent pits down to a depth of 17 m in treatments -W and +W, over 1 year before clear-cutting, then over 2 years in coppice, as well as down to a depth of 4 m in a non-harvested plot (NH) serving as a control. CO_2, CH_4 and N_2O surface effluxes were measured over three years using the closed-chamber method in treatments -W, +W and NH. CO_2, CH_4 and N_2O concentrations in the soil were measured from the pits down to a depth of 15.5 m in treatments -W, +W and NH over 3 months before the clear-cut and 1.5 years after in coppice.

After harvesting, spectacular fine root growth of trees conducted in coppice occurred in very deep soil layers (> 13 m) and, surprisingly, root mortality remained extremely low whatever the depth and the treatment. Total fine-root biomass in coppice down to a depth of 17 m was 1266 and 1017 g m^{-2} in treatments +W and -W, respectively, at 1.5 years after the clear-cut and was 1078 g m^{-2} in NH 7.5 years after planting. Specific root length and specific root area were about 15% higher in -W than in +W. CO_2, CH_4, and N_2O effluxes were not significantly different between treatments -W and +W and did not change after clear-cutting in the coppice-managed stands compared to non-harvested stand. CO_2 and CH_4 concentrations greatly increased with depth and N_2O concentrations remained roughly constant from the soil surface down to a depth of 15.5 m. Mean CO_2 and N_2O concentrations in -W were 20.7% and 7.6% lower than in +W, respectively, and CH_4 concentrations in -W were 44.4% higher than in +W throughout the soil profiles. A diffusivity model showed that CO_2, N_2O and CH_4 production and consumption occurred at great depths and were similar in treatments +W, -W and NH. Clear-cutting did not increase CO_2, CH_4 and N_2O effluxes and productions, whatever the water supply regime.

Establishing deep root systems in tropical planted forests could help trees withstand the long drought periods expected under climate change in many tropical regions. Our study suggested that coppice management might be an interesting option in tropical *Eucalyptus* plantations, both to improve tree tolerance to drought and store carbon at great depth in the soil. Consequences of climate changes on greenhouse gas emissions could be minor when tropical *Eucalyptus* plantations are coppice-managed.

Keywords: Brazil, *Eucalyptus grandis*, deep root growth, greenhouse gases, throughfall exclusion, very deep tropical soil, coppice

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RESUMO ESTENDIDO EM PORTUGUES

Resumo :

Melhorar nossa compreensão sobre a dinâmica espaço-temporal de raízes finas e dos gases de efeito estufa em camadas profundas do solo é um componente chave para identificar práticas silviculturais mais sustentáveis para florestas plantadas num contexto de mudança climática e para melhorar os atuais modelos biogeoquímicos.

Nosso estudo teve como objetivo avaliar o efeito do corte raso e da seca na produção de raízes finas, nos efluxos de CO_2, CH_4 e N_2O do solo e da produção ao longo dos perfis profundos do solo até o lençol freático nas plantações de eucalipto manejadas em talhadia. A raízes finas (diâmetro <2 mm) foram amostradas até 17 m de profundidade num experimento de exclusão de chuva comparando parcelas com 37% de precipitação excluída (-W) e sem exclusão de chuva (+ W). A dinâmica das raízes foi estudada usando minirhizotron em duas trincheiras permanentes até 17 m de profundidade nos tratamentos -W e + W, durante 1 ano antes do corte raso e 2 anos depois do corte em talhadia, e tambem até 4 m de profundidade numa parcela sem corte (NH), servindo como controle. Os efluxos de CO_2, CH_4 e N_2O na superfície do solo foram medidos ao longo de três anos utilizando o método de câmara manual nos tratamentos -W, + W e NH. As concentrações de CO_2, CH_4 e N_2O no solo foram medidas a partir das trincheiras até uma profundidade de 15.5 m nos tratamentos -W, + W e NH durante 3 meses antes do corte raso e 1.5 ano depois em talhadia.

Após o corte, ocorreu um grande crescimento radicular das árvores conduzidas em talhadia nas camadas muito profundas do solo (> 13 m) e, surpreendentemente, a mortalidade das raízes permaneceu extremamente baixa, independentemente da profundidade e do tratamento. A biomassa total de raízes finas em talhadia até 17 m de profundidade foi de 1266 e 1017 g m^{-2} em + W e -W, respectivamente, 1.5 ano após o corte raso, e 1078 g m^{-2} em NH 7.5 anos após o plantio. O comprimento específico e a área específica das raízes foram cerca de 15% maiores em -W do que em + W. Os efluxos de CO_2, CH_4 e N_2O não foram significativamente diferentes entre -W e + W e não alteraram após o corte raso nas parcelas manejadas por talhadia em comparação com o povoamento não colhido. As concentrações de CO_2 e CH_4 aumentaram muito com a profundidade e as concentrações de N_2O permaneceram aproximadamente constantes na superfície do solo até uma profundidade de 15.5 m. As concentrações médias de CO_2 e N_2O em -W foram 20.7% e 7.6% menores que em + W, respectivamente, e as concentrações de CH_4 em -W foram 44.4% maiores que em + W em todo o perfil de solo. Um modelo de difusividade mostrou que a produção e o consumo de CO_2, N_2O e CH_4 ocorreram em grandes profundidades e foram similares nos tratamentos + W, -W e NH. O corte raso não aumentou os efluxos e as produções de CO_2, CH_4 e N_2O, independentemente do regime de abastecimento de água.

O estabelecimento de sistemas radiculares profundos em florestas tropicais plantadas pode ajudar as árvores a resistir aos longos períodos de seca esperados pelas mudanças climáticas em muitas regiões tropicais. Nosso estudo sugeriu que o manejo em talhadia pode ser uma opção interessante nas plantações tropicais de eucalipto para melhorar a tolerância das árvores à seca e armazenar carbono em grande profundidade no solo. As conseqüências das mudanças climáticas nas emissões de gases de efeito estufa podem ser menores quando as plantações tropicais de eucaliptos são manejadas em talhadia.

Palavras-chave: Brazil, *Eucalyptus grandis*, crescimento de raízes profundas, gases de efeito estufa, exclusão de chuvas, solo tropical muito profundo, talhadia

Introdução

O crescimento das árvores é altamente dependente da função de absorção das raízes finas para a água e os nutrientes. Raízes finas também desempenham um papel importante no ciclo global de carbono (C), principalmente nos processos de produção, respiração, exsudação e decomposição. Melhorar a compreensão da dinâmica espaço-temporal de raízes finas ao longo de todo o perfil do solo até o lençol freático é importante para identificar práticas silviculturais mais sustentáveis para as florestas plantadas. Após uma forte expansão nos últimos 20 anos, as plantações florestais representam 7% da área florestal total do mundo (Keenan *et al.*, 2015). Em regiões tropicais e subtropicais, as plantações industriais de eucalipto de rápido crescimento, participam no armazenamento de carbono atmosférico e ajudam a reduzir a exploração das florestas naturais fornecendo um proporção crescente da demanda mundial em madeira (Keenan *et al.*, 2015). As plantações de eucalipto de rápido crescimento cobrem cerca de 20 milhões de hectares em todo o mundo (Booth, 2013), incluindo cerca de 5.67 milhões de hectares no Brasil (IBA, 2016). As florestas tropicais têm uma forte influência nas concentrações de dióxido de carbono (CO_2), óxido nitroso (N_2O) e metano (CH_4) na atmosfera. As práticas silviculturais nas florestas plantadas afetam os fluxos de gases de efeito estufa na superfície do solo e os principais determinantes da produção de gases de efeito estufa nos solos florestais (substrato, temperatura, teor de água) variam em função da profundidade do solo. No Brasil, a maioria das plantações de eucalipto são estabelecidas em solos com baixa fertilidade e submetidas a períodos de seca de até 6 meses (Gonçalves *et al.*, 2013). Apesar das projeções sobre o futuro das precipitações ainda sejam difíceis de prever em nível local, estudos mostraram que o aquecimento global será associado a uma intensificação das estações secas em muitas partes do globo (Solomon *et al.*, 2009). especialmente em regiões subtropicais já sujeitas a secas severas (Meehl *et al.*, 2007). Neste contexto, o manejo do *Eucalyptus* através da talhadia tem sido praticado por empresas do setor florestal no Brasil e pode ser uma vantagem contra o estresse hídrico, uma vez que as brotações do *Eucalyptus* já possuem um sistema radicular formado em camadas profundas, onde as quantidades de água disponíveis podem ser maiores. As conseqüências do corte raso das árvores na dinâmica radicular no primeiro ano de rotação de plantações em talhadia implicam questões específicas nunca estudadas nas camadas profundas do solo. Além disso, o corte da parte aérea das árvores tem um impacto significativo na saída de carbono do ecossistema e nas emissões de gases de efeito estufa. Após o corte, a mortalidade das raízes leva à um suprimento de matéria orgânica fresca em diferentes profundidades (Berhongaray *et al.*, 2015). A decomposição da matéria orgânica fresca pode

promover a degradação da matéria orgânica existente (Fontaine *et al.*, 2007; Derrien *et al.*, 2014) com um "efeito priming" (Kuzyakov *et al.*, 2000). O "efeito priming" pode afetar o armazenamento de carbono pelo ecossistema e afetar indiretamente o ciclo do nitrogênio. Existe pouca pesquisa sobre os ciclos biogeoquímicos em plantações florestais manejadas em talhadia, e nenhuma comparando experimentalmente estresse hídrico (exclusão de chuva) e precipitação normal. A avaliação da contribuição das raízes finas profundas e da dinâmica espaço-temporal das concentrações de gases de efeito estufa nas camadas profundas do solo é um verdadeiro desafio metrológico e uma grande barreira científica, a qual é importante levantar para estudar a sustentabilidade das plantações florestais e sua resiliência no contexto das mudanças climáticas globais. O objetivo principal da tese é avaliar o efeito do corte e da redução de chuva na produção das raízes finas, CO_2, CH_4 e N_2O ao longo do perfil do solo até o lençol freático. Estes efeitos foram acompanhados antes e após o corte raso das árvores em uma plantação de *Eucalyptus grandis* conduzida em talhadia e submetida à dois regimes hídricos contrastantes.

Os objetivos específicos da tese são:

- Melhorar a compreensão da influência das chuvas sobre a mortalidade e a produção de raízes finas em função da profundidade do solo em plantações conduzidas por talhadia;
- Avaliar o efeito do corte raso e do manejo em talhadia sobre a dinâmica das raízes finas até 17 m de profundidade;
- Avaliar a dinâmica de raízes finas, CO_2, CH_4 e N_2O próxima ao lençol freático (17 m de profundidade) em tratamentos com dois níveis de pluviosidade contrastantes;
- Determinar o perfil vertical das taxas de produção de CO_2, CH_4 e N_2O a partir da superfície até uma profundidade de 17 m, através de amostragens dos gases, seguida de análises por cromatografia gasosa e modelagem da produção e do transporte no solo.

Estratégia de pesquisa

Os experimentos deste doutorado ocorreram no Brasil, na Estação Experimental de Itatinga (Estado de São Paulo), destinada à descrição, quantificação e modelagem do funcionamento dos ecossistemas florestais tropicais. A precipitação média anual nesta área é de 1390 mm; a vegetação dominante é o eucalipto; o solo é muito profundo (> 15m) do tipo arenoso ferralítico (Laclau *et al.*, 2010). Uma plantação de *Eucalyptus grandis* de mais de dois hectares foi instalada em 2010 em um delineamento em parcelas subdivididas (split-plot) com 3 blocos. O principal fator estudado é a redução da chuva e o fator secundário é a fertilização potássica. O trabalho de tese concentra-se em modalidades com adubação potássica não limitante como em

plantações comerciais no Brasil, comparando chuvas não perturbadas e exclusão parcial por um sistema de calhas para coletar e excluir 37% das chuvas.

Duas trincheiras profundas (17 m de profundidade x 1.5 m de diâmetro) foram escavadas no início de 2014 em tratamentos com e sem exclusão de chuva e estão equipadas com plataformas que facilitam a manipulação e medições em profundidades diferentes.

As árvores foram cortadas no experimento em junho de 2016, com a idade de 6 anos, e a plantação foi conduzida em talhadia após o corte raso. No entanto, uma parcela de referência (NH) foi mantida com as árvores existentes (fertilização e chuvas não perturbadas). Nesta parcela, uma terceira trincheira de 3 m de profundidade foi escavada e preparada.

Desde sua criação em 2014, as duas trincheiras profundas foram equipadas com minirhizotrons para monitorar a dinâmica das raízes (Maeght *et al.*, 2013). Vinte e quatro tubos transparentes de 180 cm de comprimento foram instalados a 45° dentro das trincheiras permanentes (12 por trincheira) e permitiram a observação das raízes 130 cm abaixo de cada ponto de instalação (na superfície, 1.0, 3.5, 5.5, 7.5, 9.5, 11.5, 13.5 e 15.5 m). Sete tubos foram instalados na parcela de referência (superfície, 1 e 3.5 m).

A observação das raízes foi realizada por aquisição de imagem por meio de um scanner circular (CI-600 Root Growth Monitoring System, CID, EUA). Oito imagens (21.59 cm x 19.56 centímetros) no tubo foram tomadas a cada duas semanas desde Maio de 2015: um ano antes do corte das árvores e durante 2 anos após o corte das árvores, em Junho de 2016. As imagens foram analisadas usando o software WinRHIZOtron (Regent, Canadá) para traçar as raízes, marcando o comprimento e diâmetro para estimar o crescimento ao longo do tempo.

Raízes finas (diâmetro <2 mm) foram amostradas até a profundidade de 17 m para estimar a biomassa (raízes vivas) e necromassa em todo o perfil do solo, 1.5 anos após o corte nos tratamentos + W, -W e 7.5 anos após o plantio no tratamento NH.

As emissões de gases do solo foram medidas colocando um sino (diâmetro de 40 cm, altura de 10 cm) sobre uma base de amostragem instalada no local experimental. Quatro câmaras foram colocadas aleatoriamente à quatro distâncias das árvores em cada tratamento. As câmaras foram fechadas cada vez por 30 minutos. Uma alíquota de ar ambiente foié coletada com uma seringa quatro vezes consecutivas (0, 10 min, 20 min e 30 min) após o fechamento. As medições foram feitas a cada duas semanas: três meses antes do corte e 1.5 anos após o corte. Câmaras de respiração no solo (9 por tratamento e bloco) também foram conectadas à um analisador Li-Cor

8100 (Li-cor, Lincoln, Ne, EUA) para monitorar as emissões de CO_2 na superfície do solo medido a cada duas semanas. Para coletar regularmente os gases do solo (duas vezes por mês: três meses antes do corte e 1.5 anos após o corte), as trincheiras foram equipadas com seringas instaladas em profundidades (Metay *et al.*, 2007) também cobertas por imagens de minirhizotrons (0.1, 0.5, 1.0, 3.5, 7.5, 11.5 e 15.5 m). As concentrações dos gases foram determinadas por cromatografia gasosa (GC-ECD). Os dados foram aplicados à um modelo de difusividade para estimar os fluxos de CO_2, N_2O e CH_4, bem como a produção de cada camada de solo a partir das concentrações medidas nas trincheiras.

Principais resultados

O sistema de redução de chuva implantado resultou em uma diminuição significativa do conteúdo de água do solo, em média uma redução de 12.9% em -W em comparação ao + W, da superfície a uma profundidade de 16 m. Este dispositivo mostrou-se eficaz para estudar dois regimes de precipitação contrastante. Após o corte das árvores houve uma interrupção da transpiração da parte aérea, e consequentemente o solo foi recarregado com água. O teor de água no solo foi menor no tratamento -W em relação ao tratamento + W antes do corte, essa diferença entre os dois tratamentos persistiu um ano e meio após o corte.

Em primeiro lugar investigamos o impacto da redução de chuvas e do corte raso das árvores na dinâmica radicular em uma plantação de eucalipto conduzida em talhadia. Nossos resultados mostraram que as taxas diárias de crescimento radicular foram fortemente dependentes da profundidade e da estação nos tratamentos + W e -W. Após o corte das árvores, o crescimento de raízes finas começou nas camadas superiores do solo e foi crescendo ao longo do tempo em profundidade em ambos os tratamentos + W e -W. Assim, quando a plantação é conduzida em talhadia, após o corte das árvores, o início do crecimento radicular foi observado pela primeira vez 6 meses após o corte na superfície. Na profundidade 3.5-4.8 m do solo 12 meses após o corte e na profundidade 13.5-14.8 m do solo 16 meses após o corte, nos tratamentos + W e -W. As taxas diárias de crecimento alongamento das raízes atingiram o pico no final da estação seca para todas as camadas do solo nas profundidades > 6 m, quando o teor de água no solo diminuiu nas camadas superficiais. Assim, os valores diários de alongamento das raízes não foram necessariamente correlacionados com os níveis de umidade do solo para uma dada camada de solo. Observamos uma sincronização entre a diminuição da quantidade de água extraível na camada superficial e os picos de alongamento das raízes após o corte no tratamento -W. Além disso, vimos que a densidade das raízes finas reduz abaixo de 20 cm de profundidade, depois

diminui gradualmente até o lençol freático nos três tratamentos NH, + W e -W. A biomassa total de raízes finas em talhadia até 17 m de profundidade foi de 1266 e 1017 g m^{-2} em + W e -W, respectivamente, 1.5 ano após o corte raso, e 1078 g m^{-2} em NH 7.5 anos após o plantio. A densidade de raízes finas foi cerca de 25% maior em -W do que em + W e NH na maioria das camadas do solo. Em profundidades > 7 m, as densidades de raízes finas foram pelo menos duas vezes maiores em -W que em + W e NH, respectivamente.

Além disso, estávamos também interessados no efeito da redução de chuva sobre a adaptação morfológica das raízes finas em todo o perfil do solo. Nos tratamentos NH, + W e -W, mostramos que a profundidade teve pouco efeito no comprimento específico das raízes (SRL), na área específico das raízes (SRA) e no diâmetro das raízes finas. SRL e SRA foram significativamente maiores em -W que em + W, e valores semelhantes foram observados em + W e NH. Até 17 m de profundidade, os valores médios de SRL foram 28.8, 30.6 e 34.4 m g^{-1}, os valores médios de SRA de 258.8, 267.5 e 305.3 cm^2 g^{-1} e os diâmetros médios das raízes finas de 0.31, 0.32 e 0.36 mm nos tratamentos NH, + W e -W, respectivamente. Durante todo o período de estudo (3 anos) o comprimento acumulado de raízes mortas representou 6-7% do comprimento total de raízes produzido em todo o perfil do solo até 17 m de profundidade em ambos os tratamentos W e -W. Surpreendentemente, o comprimento das raízes finas mortas não aumentou após o corte raso das árvores e não diferiu entre as profundidades do solo e entre os tratamentos + W e -W.

Em seguida, analisamos o impacto do corte raso das árvores e da redução da precipitação nas emissões de gases de efeito estufa, bem como sua produção / consumo em todo o perfil do solo. Os fluxos cumulativos de CO_2 medidos nos tratamentos + W e -W foram 136.5 ± 21.8 mol m^{-2} e 130.3 ± 24.8 mol m^{-2} ano^{-1} no último ano antes do corte raso das árvores e 108.0 ± 15.8 e 119.3 ± 22.5 mol m^{-2} ano^{-1} no primeiro ano após o corte raso quando a plantação foi conduzida em talhadia. Não encontramos diferenças significativas entre as emissão de CO_2, CH_4 e N_2O entre os dois tratamentos + W e -W. Os fluxos de CH_4 foram negativos ao longo do período de estudo, de março de 2016 a dezembro de 2017, indicando um consumo líquido de CH_4 atmosférico pelo solo. Não foram observadas diferenças significativas entre os tratamentos NH e + W nas emissões de CO_2, CH_4 e N_2O mostrando nenhum efeito do corte das árvores.

Os efluxos de CO_2, CH_4, e N_2O não foram significativamente diferentes entre -W e + W e não mudaram após o corte das árvores nas parcelas manejadas em talhadia comparado às parcelas sem corte raso. As concentrações de CO_2 e CH_4 aumentaram significativamente com a

profundidade e as concentrações de N_2O permaneceram constantes da superfície a uma profundidade de 15.5 m. As concentrações médias de CO_2 e N_2O em -W foram, respectivamente, inferior de 20.7% e 7.6% a os de +W e as concentrações de CH_4 foram 44.4% superior em -W do que em + W sobre a totalidade do perfil do solo. O modelo de difusividade mostrou que a produção e o consumo de CO_2, N_2O e CH_4 ocorreu em grandes profundidades e foram semelhantes nos tratamentos + W, W, e NH. O corte raso das árvores não aumentou os efluxos e as produções de CO_2, CH_4 e N_2O independentemente do regime de água.

As produções de CO_2, CH_4, e N_2O entre 0.1 e 15.5 m de profundidade, não foram significativamente diferentes entre + W e -W. As produções mais elevadas de CO_2 e N_2O e a menor produção de CH_4 foram estimadas na superficie do solo devido ao forte gradiente de concentrações e a maior difusividade efetiva calculada na camada superficial do solo. A produção de CO_2 e N_2O e o consumo de CH_4 ocorreu principalmente na superfície e uma parte da produção de CO_2 e CH_4 atingiu uma profundidade de até 15.5 m.

Discussão geral

A principal novidade dos nossos resultados está na primeira observação direta da fenologia das raízes finas, bem como nas primeiras medições *in situ* das concentrações de CO_2, N_2O e CH_4 em profundidades à mais de 10 m em uma plantação de eucalipto conduzida em talhadia. As conseqüências do corte raso das árvores e da redução das chuvas na dinâmica da produção de raízes finas e dos gases de efeito estufa em plantações florestais levantam questões específicas que nunca foram estudadas em um perfil de solo profundo em clima tropical.

Efeito da redução de chuva

Diante de uma seca induzida, o eucalipto aumentou a densidade de raízes finas em grande profundidade. O crescimento das raízes em camadas profundas do solo pode aumentar a quantidade de água disponível necessária para o crecimento das árvores, o que poderia ser um benefício crítico para as árvores em condições severas de seca (Christina *et al.*, 2017). Após o corte, observou-se um grande crescimento das raízes à mais de 4 m de profundidade no final da temporada seca, independentemente do tratamento. O crescimento das raízes finas nas camadas profundas do solo começou quando o teor de água na camada de 0-2 m caiu abaixo de um limiar de cerca de 80 mm, sugerindo que o teor de umidade no solo, em uma determinada camada do solo, não foi o principal fator responsável pelo crescimento das raízes finas. Sucessivos afrouxamentos no crescimento radicular da superfície do solo para suas camadas mais

profundas, no final da temporada seca e nos primeiros meses após o início da temporada chuvosa nos tratamentos + W e –W, seria devido ao rápido esgotamento dos recursos hídricos superficiais, o que induziria as raízes das árvores a buscarem as camadas mais profundas do solo (Schenk and Jackson, 2002; Billings, 2015).

Além disso, mostramos que os eucaliptos em condições de seca adaptaram sua morfologia radicular para maximizar a superfície das raízes em contato com o solo para absorver os recursos limitados. Os valores de SRL e SRA foram maiores em -W que em + W, o que combinado com maiores densidades de raízes finas levaria à um índice de superfície de raiz muito maior. Nosso estudo mostrou que o eucalipto pode modificar as características das raízes finas para melhorar a captura de recursos e a exploração de camadas muitas profundas do solo, a fim de sua sobrevivência em um contexto de mudanças climáticas ajudando assim, a manter a absorção de água durante períodos secos (Brunner *et al.*, 2015; Christina *et al.*, 2017).

A exclusão da chuva não teve efeito nos fluxos de CO_2, N_2O e CH_4 medidos na superfície ou calculados em profundidade com um modelo de difusividade. As diferenças na concentração de gás no solo, medidas entre + W e -W, são provavelmente devido ao maior teor de água do solo em + W que poderia reduzir a difusividade dos gases, enquanto as emissões não foram afetadas. No tratamento + W, o alto teor de água no perfil do solo pode induzir a saturação do espaço intersticial pela água e causar uma lenta difusão dos gases de efeito estufa para a atmosfera (Maier *et al.*, 2017; Wang *et al.*, 2018). A estrutura de poros e agregados do solo está diretamente relacionada à liberação de gases de efeito estufa, mas também ao armazenamento de carbono (Smith *et al.*, 2004; Mangalassery *et al.*, 2013).

As produções de CO_2, CH_4 e N_2O não foram significativamente diferentes entre os tratamentos + W e -W até uma profundidade de 15.5 m. No entanto, observamos que a produção pode diminuir nas camadas superiores e aumentar nas camadas profundas. Nós mostramos que a biomassa de raízes e o índice de área de raiz aumentaram em aproximadamente 25% e 24%, respectivamente, no tratamento de exclusão de chuva comparado com a parcela de controle a uma profundidade de 17 m, e que a biomassa das raízes aumentou principalmente nas profundidades > 7 m. Portanto, a mudança nas produções de CO_2 nas camadas mais profundas observadas em nosso estudo pode ser devido à uma mudança nas atividades das raízes para horizontes mais profundos, indicando um aumento da captação de água na profundidade pelas raízes (Sotta *et al.*, 2007; Schwendenmann *et al.*, 2010).

Também observamos uma mudança no consumo de CH_4 nas camadas profundas no tratamento de exclusão de chuva. A mudança no consumo de CH_4 para camadas mais profundas pode ser devido às condições do solo mais favoráveis à metanotrofia, mas menos à metanogênese. Mais estudos são necessários para avaliar os efeitos da exclusão de chuva nas comunidades microbianas, suas atividades e interações, e particularmente nas camadas profundas do solo.

Efeito do corte raso das árvores

Descobrimos que poucas raízes foram perdidas pela mortalidade (<10% de todas as raízes observadas ao longo de 3 anos), sugerindo que a maior parte do sistema radicular permanece funcional após o corte raso das árvores conduzidas em talhadia. O nosso estudo sugere que árvores de *Eucalyptus grandis* de 6 anos de idade têm reservas suficientes no sistema radicular e que as condições apropriadas do solo para manter a biomassa radicular estabelecida até o lençol fréatico após o corte tenha, provavelmente, também contribuído para o crescimento inicial das rebrotas (Drake *et al.*, 2013; Brunner *et al.*, 2015).

A assincronia no sistema radicular, atrasando o crescimento das raízes nas camadas mais profundas do solo poderia ser uma estratégia para maximizar a absorção de água e nutrientes necessárias para atender a alta demanda das árvores de rápido crescimento. O crescimento das raízes ocorreu a uma profundidade de 14 m em menos de um ano após o corte das árvores conduzidas em talhadia, enquanto a profundidade máxima atingida pelas raízes de *E. grandis* é de aproximadamente 7 m um ano após o plantio em solos muito profundos (Christina *et al.*, 2011; Laclau *et al.*, 2013). As raízes profundas permitem o acesso à grandes quantidades de água armazenada no subsolo após o corte e, apesar da baixa densidade de raízes finas nas camadas mais profundas do solo, são suficientes para absorver grandes quantidades de água (Christina *et al.*, 2017). A baixa mortalidade de raízes finas após o corte sugerem que o manejo em talhadia das plantações de eucalipto pode ser uma opção promissora para lidar com a seca, uma vez que o sistema de radicular existente permite o acesso à água armazenada nos horizontes profundos.

Contrariamente às nossas expectativas, o corte das árvores não levou ao aumento de emissões e produção de CO_2, CH_4 e N_2O, independentemente do regime hídrico. A colheita de eucaliptos em plantações manejadas em rotações curtas leva a grandes alterações no ambiente do solo, provavelmente aumentando o processo de decomposição da matéria orgânica, como o aumento da temperatura do solo e umidade do solo, bem como acúmulo de resíduos de colheita na superfície do solo (Mendham *et al.*, 2002; O'connell *et al.*, 2004; Rocha *et al.*, 2016; Christina

et al., 2017). Nós mostramos que o corte das árvores não levou à um aumento da mortalidade das raízes no primeiro ano após o corte, quando a plantação foi conduzida em talhadia, independentemente do regime hídrico. Portanto, assumimos que o corte raso resultando em emissões da mesma intendisidade que antes do corte das arvores em nosso experimento pode resultar de uma diminuição na respiração das raízes após a colheita, compensa-se a um aumento na decomposição da matéria orgânica na superfície do solo.

Conclusão geral

A fenologia das raízes finas do eucalipto em talhadia submetidas à regimes hídricos contrastantes revelou taxas de mortalidade muito baixas. O crescimento inicial da parte aérea após o corte das árvores beneficiou-se do sistema radicular estabelecido na rotação anterior, e o crescimento assíncrono das raízes finas com a profundidade revelou a plasticidade das árvores em resposta às condições do solo. O estabelecimento de sistemas radiculares profundos em florestas tropicais plantadas poderia ajudar as árvores a resistir aos longos períodos de seca esperados em muitas regiões tropicais num contexto de mudanças climáticas.

Além disso, mostramos que o corte raso das árvores não aumentou significativamente as emissões de CO_2, N_2O e CH_4 quando o plantio é conduzido em talhadia em comparação com uma plantação não cortada. As emissões de gases pós-corte são consistentes com as baixas taxas de mortalidade de raízes observadas. Por outro lado, raízes profundas também poderiam contribuir para sequestrar grandes quantidades de C no solo. As alocações subterrâneas de carbono representam cerca de 20-30% da produtividade primária bruta nas plantações de eucalipto (Ryan *et al.,* 2010; Epron *et al.,* 2012; Nouvellon *et al.,* 2012). O carbono das raízes é geralmente retido por mais tempo no solo do que da serapilheira, que é mais afetada por processos físico-químicos e também devido às diferenças na composição estrutural entre as folhas e as raízes (Rasse *et al.,* 2005; Schmidt *et al.,* 2011; Menichetti *et al.,* 2015). Mais estudos são necessários para determinar se o manejo em talhadia das plantações de eucalipto em solos muito profundos pode ser uma opção efetiva para mitigar o aumento de gases de efeito estufa na atmosfera e aumentar o armazenamento de C no solo.

Solos tropicais muito profundos cobrem grandes áreas do mundo e é fundamental aumentar nossa compreensão sobre a dinâmica espaço-temporal das concentrações de gases de efeito estufa nas camadas profundas, a fim de quantificar com mais precisão fontes / armazenamento de C para estimar o orçamento global de carbono, melhorar os atuais modelos biogeoquímicos

para a previsão de emissões de gases de efeito estufa e identificar práticas silviculturais mais sustentáveis para florestas tropicais plantadas no contexto das mudanças climáticas.

RESUME ETENDU EN FRANÇAIS

Résumé

Améliorer notre compréhension de la dynamique spatio-temporelle des racines fines et des gaz à effet de serre dans les couches profondes du sol est un élément clé pour identifier des pratiques sylvicoles plus durables pour les forêts plantées dans un contexte de changement climatique et pour améliorer les modèles biogéochimiques actuels. Notre étude visait à évaluer l'effet de la coupe des arbres et de la sécheresse sur la production de racines fines et les émissions et production de CO_2, N_2O et CH_4 sur un profil de sol profond jusqu'à la nappe phréatique, dans des plantations d'*Eucalyptus* menées en taillis au Brésil. Les racines fines, d'un diamètre inférieur à 2 millimètres, ont été échantillonnées jusqu'à une profondeur de 17 m sur un dispositif d'exclusion de pluie comparant des peuplements soumis à une exclusion de 37% des pluviolessivats (-W) et des peuplements sans exclusion (+ W). La dynamique des racines a été étudiée à l'aide de minirhizotrons installés dans deux fosses permanentes d'une profondeur de 17 m dans les traitement -W et + W, pendant un an avant la coupe des arbres, puis pendant deux ans en taillis, et jusqu'à 4 m de profondeur dans un peuplement non récolté (NH) servant de témoin. Les efflux de CO_2, de CH_4 et de N_2O à la surface du sol ont été mesurés durant trois ans en utilisant la méthode des chambres manuelles dans les traitements -W, + W et NH. Les concentrations de CO_2, de CH_4 et de N_2O dans le sol ont été mesurées à partir des fosses jusqu'à une profondeur de 15.5 m dans les traitements -W, + W et NH durant 3 mois avant la coupe des arbres et 1.5 ans après, en taillis. Après la coupe, nous avons observé une croissance spectaculaire des racines fines en grande profondeur (> 13 m) chez les arbres menés en taillis et, étonnamment, une mortalité extrêmement faible des racines quelle que soit la profondeur et le traitement. La biomasse totale des racines fines jusqu'à 17 m de profondeur dans les traitements menés en taillis était de 1266 et 1017 g m^{-2} dans + W et -W, respectivement, 1.5 an après la coupe des arbres et de 1078 g m^{-2} dans le traitement NH, 7.5 ans après la plantation. La longueur spécifique et la surface spécifique des racines étaient environ 15% plus élevées dans -W que dans + W. Les efflux de CO_2, de CH_4 et de N_2O ne différaient pas significativement entre -W et + W et ne changeaient pas après la coupe des arbres dans les peuplements menés en taillis par rapport aux peuplements non exploités. Les concentrations de CO_2 et de CH_4 augmentaient fortement avec la profondeur et les concentrations de N_2O restaient à peu près constantes de la surface du sol jusqu'à une profondeur de 15.5 m. Les concentrations moyennes de CO_2 et de N_2O dans -W étaient respectivement inférieures de 20.7% et 7.6% à celles de + W et celles de CH_4 dans -W de 44.4% supérieures à celles de + W sur tout le profil de sol. Un modèle de diffusivité a montré que la production et la consommation de CO_2, de N_2O et de CH_4 se produisaient à de grandes profondeurs et étaient similaires dans les traitements + W, -W et NH. La coupe des arbres n'a pas augmenté les efflux et les productions de CO_2, de CH_4 et de N_2O, quel que soit le régime hydrique. Notre étude suggère que la gestion en taillis permet de conserver et de réexploiter le système racinaire mis en place par les Eucalyptus lors de la rotation précédente. Cette gestion est une solution pour l'atténuation des changements climatiques car elle pourrait aussi être une option intéressante d'adaptation des plantations tropicales aux changements climatiques car elle permet d'améliorer la tolérance des arbres à la sécheresse et de stocker du carbone en plus grande profondeur dans le sol.

Mots clés : Brésil, *Eucalyptus grandis*, croissance racinaire en profondeur, gaz à effet de serre, exclusion de pluie, sol tropical très profond, taillis

Introduction

La croissance des arbres dépend fortement de la fonction d'absorption des racines fines pour l'eau et les nutriments. Les racines fines jouent également un rôle important dans le cycle global du carbone (C), principalement dans les processus de production, de respiration, d'exsudation et de décomposition. Améliorer la compréhension de la dynamique spatio-temporelle des racines fines sur tout le profil de sol jusqu'à la nappe phréatique est important pour identifier des pratiques sylvicoles plus durables pour les forêts plantées.

Après une forte expansion au cours des 20 dernières années, les plantations forestières représentent 7% de la superficie forestière totale du monde (Keenan *et al.*, 2015). Dans les régions tropicales et subtropicales, les plantations d'essences à croissance rapide stockent de grandes quantités de carbone et contribuent à réduire l'exploitation des forêts naturelles en fournissant une proportion croissante de la demande mondiale en bois (Keenan *et al.*, 2015). Les plantations d'eucalyptus à croissance rapide couvrent environ 20 millions d'hectares dans le monde (Booth, 2013), dont environ 5.67 millions d'hectares au Brésil (IBA, 2016). Les forêts tropicales ont une forte influence sur les concentrations de dioxyde de carbone (CO_2), d'oxyde nitreux (N_2O) et de méthane (CH_4) dans l'atmosphère. Les pratiques sylvicoles dans les forêts plantées affectent les efflux de gaz à effet de serre à la surface du sol parce qu'elles modifient les principaux facteurs qui déterminent la production de gaz à effet de serre dans les sols forestiers (substrat, température, teneur en eau) qui varient en fonction de la profondeur.

Au Brésil, la plupart des plantations forestières sont établies dans des zones à faible fertilité du sol et caractérisées par de longues périodes de sécheresse. Les projections climatiques futures prévoient des périodes sèches plus longues et plus fortes en Amérique du Sud (Solomon *et al.*, 2009). Dans ce contexte, la gestion sylvicole en taillis des plantations d'*Eucalyptus* peut être un avantage contre le stress hydrique parce que les arbres bénéficient d'un système racinaire déjà développé et explorant les couches profondes du sol où l'eau y est plus disponible. Les conséquences de la coupe des arbres sur la dynamique des racines lors de la première année de rotation des plantations menée en taillis posent des questions spécifiques jamais étudiées, notamment sur les effets dans les couches profondes du sol. De plus, la récolte des parties aériennes des arbres a un impact important sur le bilan de carbone et les émissions de gaz à effet de serre de l'écosystème. Après la coupe, la mortalité racinaire conduit à un apport de matière organique fraiche à différentes profondeurs (Berhongaray *et al.*, 2015). La décomposition de la matière organique fraîche peut favoriser la dégradation de la matière

organique préexistante (Fontaine *et al.*, 2007; Derrien *et al.*, 2014) grâce à un "priming effect" (Kuzyakov *et al.*, 2000). Le "priming effect" pourrait affecter le stockage de carbone par l'écosystème et influer indirectement sur le cycle de l'azote. Peu d'études portent sur les cycles biogéochimiques dans des plantations forestières avec une gestion sylvicole en taillis, et aucune comparant expérimentalement un stress hydrique (exclusion de pluie) et une pluviométrie non perturbée sous climat tropical. L'évaluation de la contribution des racines fines profondes et de la dynamique spatio-temporelle des concentrations de gaz à effet de serre dans les couches profondes du sol est un véritable défi métrologique et un verrou scientifique majeur qu'il est important de lever pour étudier la durabilité des plantations forestières, ainsi que leur résilience dans le contexte des changements globaux. L'objectif principal de la thèse est d'évaluer l'effet de la coupe et d'une réduction de pluie sur la production de racines fines, de CO_2, de CH_4 et de N_2O le long du profil du sol jusqu'à la nappe phréatique. Ces effets ont été suivis avant et après la coupe des arbres dans une plantation d'*Eucalyptus* grandis menée en taillis, soumis à deux régimes hydrique contrastés.

Les objectifs spécifiques de la thèse sont :

- D'améliorer la compréhension de l'influence des précipitations sur la production et la mortalité des racines fines sur l'ensemble du profil de sol dans les plantations menées en taillis ;
- D'évaluer l'effet de la coupe et d'une gestion en taillis sur la dynamique des racines fines jusqu'à 17 m de profondeur ;
- D'évaluer la dynamique des racines fines, du CO_2, du CH_4 et du N_2O près de la nappe phréatique (17 m de profondeur) soumis à deux niveaux de précipitations contrastés ;
- Déterminer la production de CO_2, de CH_4 et de N_2O de la surface à une profondeur de 17 m

Stratégie de recherche

Les expérimentations de ce doctorat se sont déroulées au Brésil sur la station expérimentale d'Itatinga (Etat de São Paulo). Ce site expérimental est destiné à la description, la quantification et la modélisation du fonctionnement des écosystèmes forestier tropicaux. Les précipitations annuelles moyennes sur cette zone sont de 1390 mm, la végétation dominante est l'eucalyptus, le sol est très profond (> 15m) et de type ferralitique sableux (Laclau *et al.*, 2010). Une plantation d'*Eucalyptus grandis* de plus de deux hectares a été mise en place en 2010 sur un dispositif en parcelles divisées (split plot) avec 3 blocs. Le facteur principal étudié est la

réduction de pluie, et le facteur secondaire la fertilisation potassique. Les travaux de thèse se sont focalisés sur les modalités avec fertilisation potassique non limitante comme dans les plantations commerciales au Brésil, en comparant pluviométrie non perturbée et exclusion partielle par un système de gouttière permettant de récolter et d'exclure 37% des pluviolessivats.

Deux fosses profondes de 17 m de profondeur et 1.5m de diamètre ont été creusées début 2014 sur les traitements avec et sans exclusion de pluies, et équipées de paliers facilitant l'instrumentation et les mesures à différentes profondeurs.

Les arbres ont été coupés sur le dispositif en Juin 2016 à l'âge de 6 ans, et la plantation a été ensuite menée en taillis. Une parcelle de référence (NH) est toutefois maintenue avec les arbres en place (fertilisation et pluies non perturbées). Sur cette parcelle une troisième fosse de 3 m de profondeur a été creusée et instrumentée.

Depuis leur création en 2014, les deux fosses profondes ont été équipées de minirhizotrons pour le suivi de la dynamique racinaire (Maeght *et al.,* 2013). 24 tubes transparents de 180 cm de longueur ont été installés à 45° depuis l'intérieur des fosses permanentes (12 par fosse) et permettent l'observations des racines sur 130 cm en dessous de chaque point d'installation en surface, et à 1, 3.5, 5.5, 7.5, 9 .5, 11.5, 13.5, 15.5 m de profondeur. Sept tubes ont été installés dans la parcelle de référence en surface, et à 1 et 3.5 m.

L'observation des racines est réalisée par acquisition d'images par un scanner circulaire (CI-600 Root Growth Monitoring System, CID, USA). Huit images (21.59 cm x 19.56 cm) par tube sont prises toutes les deux semaines depuis Mai 2015 : pendant 1 an avant la coupe des arbres et pendant 2 ans en taillis après la coupe des arbres en Juin 2016. Les images ont été analysées en utilisant le logiciel WinRHIZO tron (Régent, Canada) pour tracer les racines en marquant leur longueur et leur diamètre afin d'estimer la croissance au cours du temps.

Les racines fines, d'un diamètre inférieur à 2 mm, ont été échantillonnées jusqu'à 17m de profondeur afin d'estimer la biomasse (racines vivantes) et la nécromasse sur l'ensemble du profil de sol à 1.5 an après coupe dans les traitements +W, -W et 7.5 ans après la plantation dans le traitement NH.

Les émissions de gaz à la surface du sol ont été mesurées en plaçant une cloche (diamètre 40 cm, hauteur 10 cm) sur une base de prélèvement installée sur le site expérimental. Quatre chambres ont été placées aléatoirement à quatre distances des arbres sur chaque traitement. Les

chambres sont fermées pendant 30 min. Une aliquote de l'air de la chambre est collecté avec une seringue, à 4 temps consécutifs (0, 10 min, 20 min et 30 min) après la fermeture. Les mesures ont été réalisé toutes les deux semaines : 3 mois avant la coupe et 1.5 ans après la coupe. Des chambres de respiration du sol (9 par traitements et par blocs) ont également été connectées à un analyseur Li-Cor 8100 (Li-cor, Lincoln, Ne, USA) permettant le suivi des émissions de CO_2 à la surface du sol, avec une mesure toutes les deux semaines. Afin de collecter régulièrement les gaz du sol, les fosses ont été équipées de micro-enceintes installées aux mêmes profondeurs que les minirhizotrons (0.1, 0.5, 1, 3.5, 7.5, 11.5 et 15.5m), et mesurées deux fois par mois depuis 3 mois avant la coupe jusqu'à 1.5 an après la coupe. Les concentrations en gaz sont déterminées par chromatographie en phase gazeuse (CPG-ECD). Un modèle de diffusivité a ensuite été utilisé à partir des données pour estimer les efflux de CO_2, N_2O et CH_4, ainsi que la production de chaque couche de sol à partir des concentrations mesurées dans les fosses.

Principaux résultats

Le dispositif de réduction de pluie mis en place a entrainé une forte diminution du contenu en eau du sol avec en moyenne une réduction de 12.9% dans -W par rapport à +W depuis la surface jusqu'à une profondeur de 16 m. Ce dispositif c'est avéré efficace pour étudier deux régimes de précipitations contrastés. Les sols se sont rechargés en eau suite à une interruption de la transpiration de la partie aérienne après la coupe des arbres. Le contenu en eau du sol était inférieur dans le traitement -W par rapport au traitement +W avant la coupe, et cette différence a persisté un an et demi après la coupe.

Dans un premier temps, nous nous sommes intéressés à l'impact de la réduction de pluie et de la coupe des arbres sur la dynamique racinaire dans une plantation d'*Eucalyptus* menée en taillis. Nos résultats ont montré que le taux journalier d'élongation racinaire était fortement dépendant de la couche de sol et de la saison dans les traitements +W et -W. En effet après la coupe des arbres, la croissance des racines fines a débuté dans les couches superficielles du sol et s'est propagé de plus en plus en profondeur au fil du temps dans les deux traitements +W et -W. Ainsi, après la coupe des arbres le démarrage des cohortes racinaires s'est d'abord observé 6 mois après la coupe en superficies, 12 mois après la coupe dans la couche 3.5-4.8 m de sol et 16 mois après la coupe dans la couche 13.5-14.8 m de sol dans les deux traitements +W et -W quand la plantation est menée en taillis. Les valeurs du taux d'élongation racinaire journalier ont culminées à la fin de la saison sèche dans toutes les couches de sol à des profondeurs

supérieures à 6 m, lorsque le contenu en eau du sol était à son minimum dans les couches superficielles. Ainsi, les valeurs d'élongation racinaire journalières n'étaient pas nécessairement corrélées avec les teneurs en eau du sol pour une couche de sol donnée. Nous avons observé une synchronisation entre la diminution de la quantité d'eau extractible dans la couche superficielle et les pics d'élongations racinaires après la coupe dans le traitement -W. De plus nous avons observés que la densité des racines fines chute en dessous de 20 cm de profondeur, puis diminue progressivement jusqu'à la nappe phréatique dans les trois traitements NH, +W et -W. La biomasse totale des racines fines jusqu'à 17 m de profondeur était de 1078 g m^{-2} dans NH, 1017 ± 301 g m^{-2} dans +W et 1266 ± 363 g m^{-2} dans -W. La densité des racines fines était environ 25% plus élevée dans -W que dans +W et NH dans la plupart des couches de sol. À des profondeurs supérieures à 7 m, les densités de racines fines étaient au moins deux fois plus élevées dans -W que dans + W et NH.

De plus, nous nous sommes intéressés à l'effet de la réduction de pluie sur l'adaptation morphologique des racines fines sur tout le profil de sol. Dans les traitements NH, +W et -W, nous avons montrés que la profondeur avait peu d'effet sur la longueur spécifique racinaire (SRL), la surface spécifique racinaire (SRA) et le diamètre des racines fines. SRL et SRA étaient significativement plus élevés dans -W que dans + W, et des valeurs similaires ont été observées dans + W et NH. Sur tout le profil de sol jusqu'à 17 m de profondeur, les valeurs moyennes de SRL étaient de 28.8, 30.6 et 34.4 m g^{-1}, les valeurs moyennes de SRA de 258.8, 267.5 et 305.3 cm^2 g^{-1} et les diamètres moyens des racines fines de 0.31, 0.32 et 0.36 mm dans les traitements NH, +W et -W respectivement. Sur toute la période d'étude (3 ans), la longueur cumulée de racines mortes ne représentait que 6-7% de la longueur cumulée de racines produite sur tout le profil de sol jusqu'à 17 m de profondeur dans les deux traitements +W et -W. Etonnamment, la longueur des racines fines mortes n'a pas augmenté après la coupe de la plantation et n'était pas différentes entre les profondeurs de sol et entre les traitements +W et -W.

Ensuite, nous nous sommes intéressés à l'impact de la coupe des arbres et d'une réduction de précipitation sur les émissions de gaz à effet de serre ainsi que sur leur production/consommation sur tout le profil de sol. Les efflux cumulés de CO_2 mesurés dans les traitements +W et -W étaient respectivement de 136. 5 ± 21.8 mol m^{-2} et de 130.3 ± 24.8 mol m^{-2} an^{-1} la dernière année avant la coupe des arbres et de 108.0 ± 15.8 et de 119.3 ± 22.5 mol m^{-2} an^{-1} la première année après la coupe quand la plantation est menée en taillis. Nous avons observé aucunes différences significatives entre les cumuls d'émissions de CO_2, CH_4 et N_2O

entre les deux traitements +W et -W. Les efflux de CH_4 ont été négatifs pendant toute la période d'étude de mars 2016 à décembre 2017, indiquant une consommation nette de CH_4 atmosphérique par le sol. Aucunes différences significatives ont également été observés entre les traitements NH et +W sur les émissions de CO_2, CH_4 et N_2O, signifiant que la coupe des arbres n'a eu aucun effet.

Les efflux de CO_2, de CH_4 et de N_2O ne différaient pas significativement entre -W et +W et ne changeaient pas après la coupe des arbres dans les peuplements menés en taillis par rapport aux peuplements non exploités. Les concentrations de CO_2 et de CH_4 augmentaient fortement avec la profondeur et les concentrations de N_2O restaient à peu près constantes de la surface du sol jusqu'à une profondeur de 15.5 m. Les concentrations moyennes de CO_2 et de N_2O dans -W étaient respectivement inférieures de 20.7% et 7.6% à celles de +W et celles de CH_4 dans -W de 44.4% supérieures à celles de +W sur tout le profil de sol. Un modèle de diffusivité a montré que la production et la consommation de CO_2, de N_2O et de CH_4 se produisaient à de grandes profondeurs et étaient similaires dans les traitements +W, -W et NH. La coupe des arbres n'a pas augmenté les efflux et les productions de CO_2, de CH_4 et de N_2O, quel que soit le régime hydrique.

Les productions de CO_2, CH_4 et N_2O entre 0.1 et 15.5 m de profondeur n'étaient pas significativement différentes entre +W et -W. Les estimations de production dans le premier mètre de sol ont montré les valeurs les plus élevée de CO_2 et de N_2O et les plus faibles de CH_4 en raison d'un fort gradient de concentration et d'une diffusivité effective calculée particulièrement élevée pour la couche de sol superficielle. La production de CO_2 et de N_2O et la consommation de CH_4 se sont produites principalement dans le premier mètre, mais au moins une partie de la production de CO_2 et de CH_4 a atteint une profondeur allant jusqu'à 15.5 m.

Discussion générale

Dans cette thèse, nous avons réalisé pour la première fois des mesures in situ de phénologie des racines fines et de concentrations de CO_2, N_2O et CH_4 à des profondeurs de plus de 10 m dans une plantation d'eucalyptus menée en taillis. Les conséquences de la coupe des arbres ainsi qu'une réduction de pluie sur la dynamique de production des racines fines et des gaz à effet de serre dans des plantations menée en taillis soulèvent des questions spécifiques qui n'ont jamais étudiées auparavant sur un profil de sol profond en climat tropical.

Effet de la réduction de pluie

Face à une sécheresse induite, les *Eucalyptus* ont augmenté la densité de leurs racines fines en grande profondeur. La croissance des racines dans les couches profondes du sol augmente l'espace exploré par les arbres, et peut donc augmenter la quantité d'eau disponible nécessaire pour assurer leur croissance. Cet effet pourrait constituer un avantage essentiel pour les arbres en cas de sécheresse intense (Christina *et al.*, 2017). Après la coupe, une croissance racinaire spectaculaire a été observé à plus de 4 m de profondeur à la fin de la saison sèche, peu importe le traitement. La croissance des racines fines dans les couches profondes du sol a commencé lorsque la teneur en eau dans la couche de sol de 0-2 m est tombée en dessous d'un seuil d'environ 80 mm, ce qui suggère que la teneur en eau du sol dans une couche de sol particulière n'était pas le principal facteur responsable de la croissance des racines fines. Les flushs successifs de croissance des racines de la couche superficielle jusqu'aux couches les plus profondes du sol à la fin de la saison sèche et dans les premiers mois qui ont suivi le début de la saison des pluies dans les traitements + W et -W serait dus à un épuisement rapide des ressources en eau de la couche superficielle, qui induirait les racines des arbres à s'enfoncer plus profondément dans le sol (Schenk and Jackson, 2002; Billings, 2015).

De plus nous avons montrés que les eucalyptus confrontés à la sécheresse ont adapté leur morphologie racinaire afin de maximiser la surface racinaire en contact avec le sol pour absorber les ressources limitées. Les valeurs de SRL et SRA étaient plus élevées dans –W par rapport à +W, ce qui, combiné à des densités de racines fines plus élevées, conduirait à un indice de surface racinaire beaucoup plus élevé. Notre étude a montré que les eucalyptus peuvent modifier les traits des racines fines pour améliorer la capture des ressources et l'exploration de couches de sol très profondes afin de survivre dans un contexte de changement climatique en contribuant au maintien de l'absorption d'eau pendant les périodes sèches (Brunner *et al.*, 2015; Christina *et al.*, 2017).

De plus, l'exclusion de pluie n'a eu aucun effet sur les efflux de CO_2, N_2O et CH_4 mesurés à la surface ou calculés en profondeur avec le modèle de diffusivité. Les différences de concentration de gaz dans le sol, mesurées entre +W et –W proviennent probablement de la teneur plus élevée en eau dans le sol en +W, ce qui pourrait par la suite réduire la diffusivité des gaz du sol alors que les émissions n'ont pas été affectés. Dans le traitement +W, la teneur élevée en eau dans le profil de sol pourrait induire une saturation de l'espace interstitiel par l'eau et entraîner une lente diffusion des gaz à effet de serre vers l'atmosphère (Maier *et al.*, 2017; Wang *et al.*, 2018). La structure des pores et des agrégats du sol est directement liée à la

libération de gaz à effet de serre mais également au stockage de carbone (Smith *et al.*, 2004; Mangalassery *et al.*, 2013).

La production de CO_2, de CH_4 et de N_2O n'était pas significativement différente entre les traitements +W et -W jusqu'à une profondeur de 15.5 m. Cependant, nous avons observé que la production peut diminuer dans les couches supérieures et augmenter dans les couches profondes. Nous avons montré que la biomasse racinaire ainsi que l'indice de surface racinaire augmentaient d'environ 25% et 24% respectivement dans le traitement d'exclusion de pluie par rapport à la parcelle témoin jusqu'à une profondeur de 17 m, et que la biomasse racinaire augmentait principalement à des profondeurs supérieures à 7 m. Par conséquent, le déplacement de la production de CO_2 vers la couche plus profonde observé dans notre étude pourrait être dû à un déplacement de l'activité racinaire vers les horizons plus profond, indiquant une augmentation de l'absorption d'eau par les racines profondes (Sotta *et al.*, 2007; Schwendenmann *et al.*, 2010).

Nous avons également observé un déplacement de la consommation de CH_4 vers les couches profondes du traitement d'exclusion de pluie. Le déplacement de la consommation de CH_4 vers les couches plus profondes pourrait être dû à des conditions de sol plus favorables à la méthanotrophie mais défavorable à la méthanogénèse. Des études complémentaires sont nécessaires pour évaluer les effets de l'exclusion de pluie sur les communautés microbiennes, leur activité ainsi que leurs interactions, en particulier dans les couches profondes du sol.

Effet de la coupe des arbres

Nous avons mis en évidence que relativement peu de racines ont été perdues par mortalité, avec moins de 10% de mortalité sur toutes les racines observées sur 3 ans, ce qui suggère que la plus grande partie du système racinaire reste fonctionnelle après la coupe des arbres quand la plantation est menée en taillis. Notre étude suggère que les *Eucalyptus grandis* âgés de 6 ans disposent de réserves suffisantes dans le système racinaire pour maintenir la biomasse racinaire établie jusqu'à la nappe phréatique après la coupe, ce qui a probablement contribué à la croissance précoce des rejets (Drake *et al.*, 2013; Brunner *et al.*, 2015).

L'asynchronisme au sein du système racinaire, retardant la croissance des racines dans les couches profondes du sol, pourrait être une stratégie employée pour maximiser l'absorption d'eau et de nutriments nécessaire pour répondre à la forte demande de ces arbres à croissance rapide. La croissance des racines s'est produite à une profondeur de 14 m moins d'un an après

la coupe des arbres quand ils sont menés en taillis, tandis que la profondeur maximale atteinte par les racines de *E. grandis* est d'environ 7 m un an après la plantation dans des sols très profonds (Laclau *et al.*, 2013; Christina *et al.*, 2017). Les racines profondes permettent un accès à de grandes quantités d'eau stockées dans le sous-sol après la coupe et malgré la faible densité de racines fines dans les couches profondes du sol, elles suffisent à absorber des quantités importantes d'eau (Christina *et al.*, 2017). Les faibles taux de mortalité des racines fines après la coupe suggèrent que la gestion en taillis des plantations d'Eucalyptus peut être une option prometteuse pour faire face à la sécheresse, étant donné que le système racinaire préexistant permet un accès à l'eau stockée dans les horizons profonds.

Contrairement à ce que nous espérions, la coupe des arbres n'a pas entraîné d'augmentation des émissions et de la production de CO_2, de CH_4 et de N_2O, quel que soit le régime hydrique. La récolte de la biomasse aérienne entraîne une réduction de l'absorption de carbone par la photosynthèse immédiatement après la coupe, mais également des modifications substantielles de l'environnement physique du sol, telles qu'une augmentation de la température (Smethurst and Nambiar, 1990) et de l'humidité (Cortina and Vallejo, 1994), qui influencent les processus de décomposition. En effet, nous avons montrés que la coupe des arbres n'entraînait pas d'augmentation de la mortalité racinaire la première année après la coupe lorsque la plantation était menée en taillis. Par conséquent, nous supposons que la décomposition des racines mortes ne s'est pas accélérée après la coupe. Nous pouvons supposer que la diminution de la respiration des racines après la coupe, associée à l'augmentation de la décomposition des litières à la surface du sol se compensent, entraînant des émissions similaires à celles d'avant la coupe.

Conclusion générale

La phénologie des racines fines d'eucalyptus menée en taillis soumis à des régimes hydriques contrastés a révélé des taux de mortalité très faible. La croissance précoce des pousses après la coupe des arbres a bénéficié du système racinaire établi au cours de la rotation précédente, et l'asynchronisme de la croissance des racines fines en fonction de la profondeur a mis en évidence la plasticité des arbres en réponse aux conditions du sol. La mise en place de systèmes racinaires profonds dans les forêts tropicales plantées pourrait aider les arbres à résister aux longues périodes de sécheresse attendues dans de nombreuses régions tropicales dans un contexte de changement climatique.

De plus, nous avons montré que la coupe des arbres n'entraine pas de manière significative l'augmentation des émissions de CO_2 et de CH_4 quand la plantation est menée en taillis par

rapport à une plantation non exploitée. Les émissions de gaz après la coupe sont cohérentes avec les faibles taux de mortalité racinaire observés. D'autre part, les racines profondes pourraient également contribuer à la séquestration de grandes quantités de C dans le sol. Les allocations de carbone vers les parties souterraines représentent environ 20 à 30% de la productivité primaire brute dans les plantations d'*Eucalyptus* (Ryan *et al.*, 2010; Epron *et al.*, 2012; Nouvellon *et al.*, 2012). Le C des racines est généralement retenu plus longtemps dans le sol que celui de la litière aérienne, qui est davantage affectée par les processus physico-chimiques et qui comporte une composition structurelle différente (Rasse *et al.*, 2005; Schmidt *et al.*, 2011; Menichetti *et al.*, 2015). Des études complémentaires sont nécessaires pour déterminer si la gestion des plantations d'eucalyptus dans des sols très profonds pourrait constituer une option efficace pour atténuer l'augmentation du CO_2 dans l'atmosphère et augmenter le stockage du C.

Les sols tropicaux très profonds couvrent de vastes régions du monde et il est essentiel d'améliorer notre compréhension de la dynamique spatio-temporelle des concentrations de gaz dans les couches profondes du sol afin de quantifier plus précisément les source / puits de C pour estimer le budget global du carbone, améliorer les modèles biogéochimiques actuels pour la prédiction des émissions de gaz à effet de serre, et identifier des pratiques sylvicoles plus durables pour les forêts tropicales plantées dans le contexte des changements climatiques.

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UNESP	Universidade Estadual Paulista Júlio de Mesquita Filho
FCA	Faculdade de Ciências Agronômicas
ESALQ	Escola Superior de Agricultura Luiz de Quieroz
USP	São Paulo State University
CIRAD	Centre de coopération internationale en recherche agronomique pour le développement
-W	Throughfall exclusion treatment
+W	Treatment with undisturbed rainfall
WFPS	Water filled pore space
GDP	Gross domestic product
GHG	Greenhouse gases
SWC	Soil water content
LP	Length production
CLP	Cumulative length production
RER	Root elongation rate
Mean RER	Mean of root elongation rate
Max RER	Maximum root elongation rate
DRER	Daily root elongation rate
SRL	Specific root length
SRA	Specific root area
FRD	Fine root density
FRB	Fine root biomass
RAI	Root area index
RLI	Root length index
HSD	Tuckey's post-hoc Honest Significant Difference
Min3P	The Reactive Transport Code MIN3P: Multicomponent reactive transport modeling in variably saturated porous media

Table 1: Summary of the root parameters used in this study, how we obtained them and how we can interpret them.

How to measure	Root parameter		Unit	All roots / Individual roots	Description	Interpretation
Minirhizotron	LP	Length production	cm m^{-2}	Individual root	Individual root length per observed soil area	Root length observed
	CLP	cumulative length production	cm m^{-2}	Σ roots	Sum of LP	Total root length observed
	RER	root elongation rate	cm day^{-1}	Individual root	Root increment between two sessions over time	Individual root growth
	Max RER	Maximum root elongation rate	cm day^{-1}	Comparing all roots	Higher value	Maximum root growth observed
	Mean RER	Mean Root elongation rate	cm day^{-1}	Taking into account all roots	Mean root elongation rate over the study period	Mean root growth
	DRER	Daily root elongation rate	cm m^{-2} day^{-1}	Σ roots	Sum of root elongation rate per session	Total root increment per day => indicator of phenology
Root sampling	FRB	Fine root biomass	g kg^{-1}	All roots	g of roots per kg of soil	Indicator of root distribution in soil
	FRD	Fine root density	g dm^{-3}	All roots	g of roots per dm^{-3} of soil sampled	Indicator of root distribution in soil
	SRL	Specific root length	m g^{-1}	All roots	Length of roots per g of root	Indicator of root exploration and nutrient uptake
	SRA	Specific root area	m^2 g^{-1}	All roots	Area of root per g of root	Indicator of root exploration and nutrient uptake
	RLI	Root length index	m m^{-2}	All roots	Length of root per soil area	Indicator of root investment
	RAI	Root area index	m^2 m^{-2}	All roots	Area of root per soil area	Indicator of root investment

LIST OF SYMBOLS

C	Carbon
N	Nitrogen
K	Potassium
P	Phosphor
CO_2	Carbon dioxide
N_2O	Nitrous oxide
CH_4	Methane
NH_4^+	Ammonium
NO_3^-	Nitrate
$CaCO_3$	Calcium carbonate

CHAPTER 1: INTRODUCTION

Many studies have been carried out other the last decades to help facing future dramatic climatic and demographic changes (Vogt *et al.*, 1995; Alexandratos, 1999; Cassman, 1999). The research work of the IPCC (Intergovernmental Panel on Climate Change), created in 1988, highlights the impact of climate change, mainly anthropogenic, on ecosystems (Metz *et al.*, 2007). The Kyoto treaty was signed in 1997 to fight against the increase in the atmospheric concentration of greenhouse gases. The increase of carbon dioxide (CO_2) and other greenhouse gases (GHG) contents in the atmosphere, mainly methane (CH_4) and nitrous oxide (N_2O), and their role in global warming are undeniable (Graefe *et al.*, 2008; Solomon *et al.*, 2009). Reducing those emissions to avoid a temperature increase of more than 2°C above pre-industrial era is a real challenge recognized by the entire scientific community (IPCC, 2014). In this perspective, the emission of greenhouse gases into the atmosphere can be directly reduced (or offset) by the sequestration of carbon in some compartments participating in its cycle. Soil represents the largest reservoir of continental organic carbon (Jacobson *et al.*, 2000). It is estimated that soil sequester approximately 800 Gt over the first 30 cm and about 1500 Gt when considering the first meter (Post *et al.*, 1982; Eswaran *et al.*, 1993; Batjes, 1996). Therefore, a tiny variation of those stocks can have a strong effect on the global atmospheric carbon. In this context, the French Minister of Agriculture launched the "4 per 1000" initiative whose objective is to develop agronomic research in order to increase soil organic carbon stocks by 4‰ per year. Such an increase would make it possible to offset greenhouse gases emissions and mitigate climate change (Lal, 2016; Chabbi *et al.*, 2017; Dignac *et al.*, 2017; Minasny *et al.*, 2017; Corbeels *et al.*, 2019). Agriculture is the first source of greenhouse gas emissions (FAO, 2015) when combined with land-use change and forestry, which both represent approximately 17.4% of worldwide GHG emissions. While many studies have been carried out to estimate the carbon balance in boreal and temperate forests (Falge *et al.*, 2002; Baldocchi *et al.*, 2005), comprehensive studies quantifying the main fluxes of the C cycle are scarce in tropical forest ecosystems. More study cases accounting for the interactive effects of (future) climate, soil, and management practices are needed in tropical regions to improve global biogeochemical models (Penuelas *et al.*, 2013). Hence, it is important to assess the feasibility, timeliness, and effectiveness of a potential use of planted forests as a strategy to mitigate greenhouse gas emissions.

I.1 Forest plantations

The area of forest plantations has increased sharply in recent decades in response to the increase in global demand for timber, mainly as a result of population growth, rising living standards, and the emergence of new markets for wood products, such as the use of wood as a substitute for fossil fuels (e.g. iron and steel industry). A study based on the Global Forest Products Model (Buongiorno *et al.*, 2003) predicts a 20% increase in world demand for wood by 2060, mainly driven by the increasing consumption of paper and wood for buildings (Elias and Boucher, 2014). While natural forests areas decreased from 3961 million hectares in 1990 to 3721 million hectares in 2015, forest plantation areas increased from 168 to 278 million hectares during the same period (Keenan *et al.*, 2015; Payn *et al.*, 2015). Thus, forest plantations account for 7% of the total of forest area in the world in 2015, compared to 4.1% in 1990. In 2000, they accounted for 5% of the global forest cover but provided 33% of roundwood harvested and reached 46% in 2012, with large disparities between regions in the world: 65% in tropical and subtropical regions, 45% in temperate regions and only 14% in boreal regions. In tropical and subtropical regions, plantations of fast-growing species (mainly *Eucalyptus*, *Pinus* and *Acacia* genera) store large amounts of carbon and contribute to reducing the exploitation of natural forests by providing an increasing proportion of the global demand of wood (Keenan *et al.*, 2015), and accounted for 29% of global forest plantation areas (Payn *et al.*, 2015). Highest productivities are reached in tropical and subtropical regions. Plantation productivity is on average 4.6 m^3 ha^{-1} $year^{-1}$ and greatly vary around the world from 1.4 m^3 ha^{-1} $year^{-1}$ on average in boreal regions to 8.4 m^3 ha^{-1} $year^{-1}$ in tropical regions (Payn *et al.*, 2015). South America with an average productivity of 24 m^3 ha^{-1} $year^{-1}$ is the area with the highest productivity. Indeed, in 2012, Brazil's forest plantations, despite an area of only 7.7 million hectares, produced 132 million m^3, as much as the United States with an area of 79 million hectares that produced 141 million m^3.

I.2 *Eucalyptus* plantations

Fast-growing *Eucalyptus* plantations (Figure I. 1) cover about 20 million hectares throughout the world (Booth, 2013) and are the most planted tree species in Brazil with about 5.6 million hectares, which represents 72.3% of the area of Brazilian forest plantations and 81% of the Brazilian wood production (IBA, 2016). *Eucalyptus* plantations play an important role in the supply of pulp, paper and power generation in Brazil, accounting for 5% of the country's GDP, in addition to 2.4 million direct and indirect jobs (IBA, 2016). These forests of great economic

interest have a mean annual increment of 40 m^3 ha^{-1} $year^{-1}$ on rotations of 6-8 years, ranging between 25 and 60 m^3 ha^{-1} $year^{-1}$ depending on forest management practices and regions (Oliveira *et al.*, 1999). With a gross primary productivity that can exceed 3500 g C m^{-2} $year^{-1}$ (Ryan *et al.*, 2010; Nouvellon *et al.*, 2012), *Eucalyptus* plantations in Brazil are among the most productive forests in the world (Luyssaert *et al.*, 2007). The significant productivity gains over the last 25 years, from 25 m^3 ha^{-1} $year^{-1}$ in the early 1990s to more than 40 m^3 ha^{-1} $year^{-1}$ nowadays, resulted from improvements in silvicultural practices and breeding programs making it possible to identify highly performant clones from some *Eucalyptus* species and hybrids, in particular *E. grandis*, *E. urophylla*, *E. saligna* and *E. camaldulensis*. The diversity of *Eucalyptus* species planted in tropical regions has led to a wide range of products and management practices (Gonçalves *et al.*, 2013). Although many *Eucalyptus* plantations are intensively managed to produce raw materials for industry (mainly pulp and paper, but also solid-wood products, fiberboards and charcoal for steel production), energy production from wood for domestic use also contributes to alleviating poverty in developing countries (Cossalter and Pye-Smith, 2003; Nambiar and Harwood, 2014). The development of industries that use wood as raw materials has generated more attention from researchers to develop new techniques oriented towards the increase of productivity and wood quality. Highly productive industrial plantations are generally perceived as a threat to biodiversity, water resources or soil fertility. The environmental impact of those highly productive forest plantations has raised many concerns in recent decades (Cossalter and Pye-Smith, 2003). Nevertheless, the problems associated with these plantations are often site-specific, and how they are managed is of paramount importance.

Figure I. 1: *Eucalyptus* plantation located in the southeastern region of Brazil, in São Paulo state.

I.3 Consequences of climate change on the forest plantations management

In Brazil, most of *Eucalyptus* plantations are settled in areas with very deep soils (> 5m), low soil fertility and prolonged drought periods (Gonçalves *et al.*, 2013). Even if future precipitation distributions are still difficult to predict at the local scale, studies have shown that global warming will be associated with an intensification of dry periods in many parts of the globe (Figure I. 2)(Solomon *et al.*, 2009), especially in sub-tropical areas already subject to severe droughts (Meehl *et al.*, 2007). A throughfall exclusion experiment in the Amazon showed that a decrease in precipitation can have a considerable influence on the functioning of forest ecosystems (Brando *et al.*, 2008). The dynamics of fine roots, litter fall, soil organic matter decomposition and nutrient mineralization, as well as soil aeration (affecting the diffusion of gases and microbial processes in the soil) are highly sensitive to rainfall (Van Straaten *et al.*, 2010; Maier *et al.*, 2017). In this context, it is important to better understand the factors influencing the response of forest ecosystems and their ability to improve their adaptation to drought conditions (Choat *et al.*, 2012).

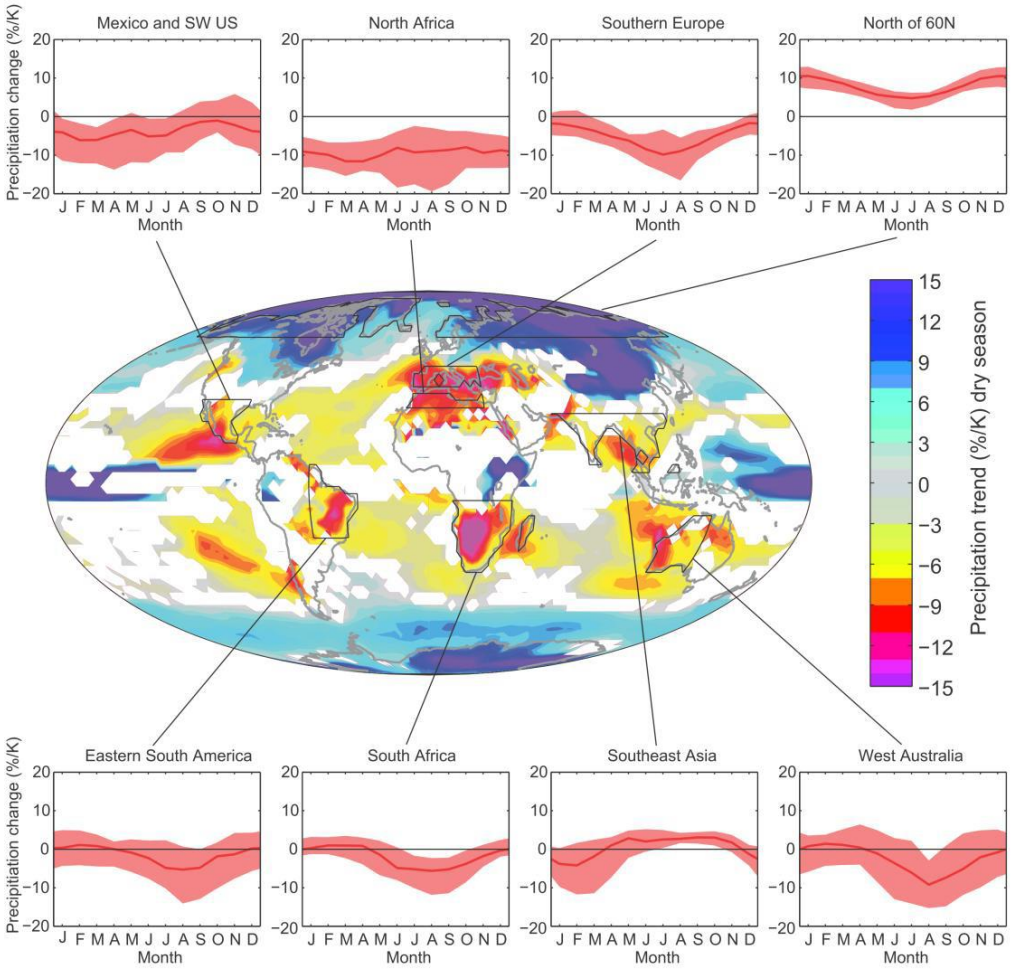

Figure I. 2: Average forecasts of changes in global rainfall distribution. Rainfall variations are expressed as a percentage change in precipitation per degree of warming. Predictions are made for decadal precipitation variations from 1900-1950 as the reference period (Solomon *et al.*, 2009).

In tropical and subtropical regions, the productivity of intensively-managed *Eucalyptus* plantations is mainly controlled by water availability in the soil (Smethurst, 2010; Gonçalves *et al.*, 2013). The management of fast-growing *Eucalyptus* plantations should be adapted in the future to more frequent drought conditions to avoid tree mortality (White *et al.*, 2009; Battie-Laclau *et al.*, 2014b; White *et al.*, 2014). The selection of drought-tolerant clones and hybrids will be necessary to reduce tree water requirements, as well as a possible adjustment in planting density, fertilization regime and rotation duration (Booth, 2013).

Furthermore, the main criticism of industrial *Eucalyptus* plantations is their high-water consumption, which can affect the water resources of the regions concerned by large areas of afforestation (Jackson *et al.*, 2005). Soil moisture monitoring down to a depth of 10 m in Brazilian eucalypt plantations showed no drainage beyond the root system, from the third year after planting onward, even though the annual precipitations was relatively high (about 1400

mm year^{-1}) (Christina *et al.*, 2011; Laclau *et al.*, 2013). Silvicultural management also has a significant impact on the water cycle. The clear-cut of the trees allows a recharge of the deep horizons of the soil with a rise of several meters of the water table (Almeida *et al.*, 2007; Christina *et al.*, 2017). Improving our understanding of soil water transfers after cutting the trees and their impact on the microbial processes involved in C and nutrient cycling is important for the management of these planted forests in a context of climate change (Sheffield and Wood, 2008; Solomon *et al.*, 2009; IPCC, 2014).

I.4 The importance of fine roots for tree growth

Being the main organ for water and nutrient uptake by plants, fine roots (diameter ≤ 2 mm) play an essential role in plant growth (Hinsinger, 2001; Brunner *et al.*, 2013; Upson and Burgess, 2013). The establishment of a deep root system can be a strategy for plant species to adapt to water stress. In poor soils, most of the fertility is concentrated in the surface horizon as a result of deposition and decomposition of aboveground litter, soil organic matter mineralization and fine root decomposition. The high root growth rate of *Eucalyptus* trees allows them to explore both the superficial soil layers and very deep soil horizons at early stages. Fine roots in eucalypt plantations can reach a depth of 7 m the first year after planting when there is no barrier for root growth (Christina *et al.*, 2011; Laclau *et al.*, 2013). However, the relative contribution of deep roots to mineral nutrition is still poorly documented in these plantations (Da Silva *et al.*, 2011; Bordron *et al.*, 2018).

Plant species use a large range of rooting patterns to cope with periodic drought, from "drought tolerant strategies" with fine roots surviving in periodically dry soil, to "drought avoiding strategies" shedding fine roots from dry soil horizons while rapidly developing roots in moister areas (Brunner *et al.*, 2015; Vries *et al.*, 2016; Bristiel *et al.*, 2018). Drought can increase the root-to-shoot ratio, the root area or root length-to-leaf area ratio, as well as the proportion of fine roots in deep soil layers and/or the specific root area (Markesteijn and Poorter, 2009; Ma *et al.*, 2018; Zhou *et al.*, 2018). Root growth peaks have been shown in deep soil layers during or the first months just after dry periods for eucalypt and rubber trees in tropical soils (Maeght *et al.*, 2015; Lambais *et al.*, 2017). Drought tolerance strategies are common for herbaceous plants, while drought avoiding strategies are often adopted by trees in evergreen tropical forests (Brunner *et al.*, 2015). Despite the crucial role of fine roots to cope with drought, root phenology remains poorly understood in comparison to leaf ecophysiology (Radville *et al.*, 2016).

Fine root dynamics and turnover depend on the plant species, but also on biotic and abiotic parameters. For example, soil water contents and soil properties, such as pH, nitrogen and oxygen concentrations can strongly influence fine root dynamics (Vogt *et al.*, 1995; Satomura *et al.*, 2007; Guo *et al.*, 2008). Indeed, observations in *Eucalyptus* plantations suggest a link between the vertical distribution of fine roots and nutrient acquisition by the trees (Bouillet *et al.*, 2002; Da Silva *et al.*, 2011; Laclau *et al.*, 2013), which can itself develop a high plasticity of its root system to colonize preferentially nutrient-rich areas (Hodge, 2004).

I.5 How to study fine root dynamics

There is no consensus on the best method to measure fine root production in forest ecosystems, and most studies conclude that a combination of different methods gives the most reliable results (Vogt *et al.*, 1995; Hertel and Leuschner, 2002; Hendricks *et al.*, 2006; Jourdan *et al.*, 2008). Successive soil sampling with a sequential coring measurement is the most commonly used method to study fine root production in natural or planted forest ecosystems (Persson, 1978; Mello *et al.*, 2007; Jourdan *et al.*, 2008). It is frequently associated with the installation of "ingrowth cores", both techniques are based on destructive soil samplings. In the last method, new fine roots produced in a root-free soil volume are regularly collected and quantified within mesh bags every month or more, during an entire year, to estimate the annual fine root production (Hendricks *et al.*, 2006). However, several studies have shown that soil disturbances (in particular fine root cutting) that occurred during the installation of the mesh bags significantly changes fine root dynamics (Vogt *et al.*, 1998; Hertel and Leuschner, 2002; Jourdan *et al.*, 2008). Since the 1980s, the minirhizotron technique is widely used to study fine root dynamics and turnover (Withington *et al.*, 2003; Graefe *et al.*, 2008; Maeght *et al.*, 2013). This technique allows direct observation and measurement of fine root dynamics while minimizing soil disturbance if a long period of recovery is observed prior to root measurements. It is a non-destructive method that involves the installation of transparent tubes, called minirhizotrons, in the soil near the studied plants (Majdi *et al.*, 2001; Tierney and Fahey, 2001; Withington *et al.*, 2003). With this transparent interface, fine roots can be measured at successive dates to study the dynamics of growth and mortality (Hendrick and Pregitzer, 1996; Anderson *et al.*, 2003). The opportunity to study fine roots in a mildly disturbed environment is an important asset of this method (Majdi *et al.*, 2001).

I.6 Specificities of fine roots in deep soil layers

Deep fine roots are fundamental organs for the growth and survival of plants. They help to avoid nutrient losses through deep drainage (Laclau *et al.*, 2010; Mareschal *et al.*, 2013), and to withdraw water at great depth during dry periods (Schwendenmann *et al.*, 2010; Battie-Laclau *et al.*, 2014b; Christina *et al.*, 2015). Fine root dynamics at the vicinity of very deep water tables have been little studied (Soylu *et al.*, 2014), mainly due to technical difficulties, although the fine root behavior can be essential for plant survival during severe drought events (Nepstad *et al.*, 2007; Malhi *et al.*, 2009; Markewitz *et al.*, 2010; Ivanov *et al.*, 2012). Fine root lifespan depends on many factors such as root order (Guo *et al.*, 2008), diameter (Joslin *et al.*, 2006) and soil depth (Hendrick and Pregitzer, 1996; Baddeley and Watson, 2005; Germon *et al.*, 2016). Most of the studies dealing with fine root production and turnover have been carried out in the upper soil layers (depth <1 m) mainly due to the difficulty to realize such measurements at great depth (Billings, 2015). Improving our understanding of water and nutrient uptake processes at great depth will become more important for forest management in a context of climate change since trees will be more (Nepstad *et al.*, 1994; Malhi *et al.*, 2009).

Studies carried out in Brazil showed that about 10% of the total fine root biomass is distributed below 3 m of depth in eucalypt plantations (Christina *et al.*, 2011). Laclau *et al.* (2013) showed that the proportion of fine roots below 4 m deep increased with the age of *Eucalyptus* trees: fine roots below 4 m deep represented 5%, 10% and 20% of the fine root intersects on vertical pit walls in plantations of 1, 3.5 and 6 years old, respectively. In Brazil, the first estimates of the production and turnover of *Eucalyptus* fine roots were conducted in topsoil horizons limited to a depth of 1 m (Mello *et al.*, 2007; Jourdan *et al.*, 2008), while little is known about these processes at depth. Lambais *et al.* (2017) studied root production and mortality during the first rotation of *Eucalyptus* plantation down to a depth of 6 m. They showed that *Eucalyptus* fine roots colonized quickly the very deep soil horizons (depth > 5 m) and that the root elongation rate was higher between the depths of 5 and 6 m compared to the top soil from 2 to 4 years after planting. In addition, there was only 3.4% of root length lost by mortality in the 5-6 m soil layer during the 2 years of study, whereas 22.7% of the fine roots had died in the superficial layer. There is little quantitative data on deep fine root dynamics (> 6 m) and literature tends to underestimate the depth of the root system distribution as well as its importance in ecosystems functioning and productivity (Schenk and Jackson, 2002; Markewitz *et al.*, 2010; Freycon *et al.*, 2015).

I.7 Relationships between fine root dynamics and GHG fluxes

Fine roots participate actively in the C fluxes in forest soils through the emission of C to atmosphere by respiratory processes and C storage in the soil from root mortality and exudation of carbonic compounds (Balesdent and Balabane, 1996; Strand *et al.*, 2008). Then, it is challenging to compare the observations of fine root production and mortality with the dynamics of CO_2, CH_4 and N_2O throughout deep soil profiles in the context of climate change. *Eucalyptus* trees allocate about 30% of their gross primary productivity to root maintenance and production in highly productive plantations managed in short rotations (Ryan *et al.*, 2010), but the difficulty to estimate fine root dynamics throughout the entire soil profile limits the estimation of the global soil carbon cycle and the total emissions of greenhouse gases (GHG), as well as the understanding of factors controlling them (Wang *et al.*, 2013).

Greenhouse gases (GHG) emissions from soils are the result of complex production, consumption and transport processes (Figure I. 3), and are affected by many factors such as microclimate, substrate availability for soil micro-organisms, aboveground photosynthetic supply to roots and management factors (Metay *et al.*, 2007). CO_2 effluxes at soil surface come from root respiration, anaerobic and aerobic microbial respiration and soil organic matter decomposition (Figure I. 3) (Versini *et al.*, 2013; Oertel *et al.*, 2016). CH_4 effluxes depend on the balance between production by methanogenesis under anaerobic conditions and consumption by methanotrophic microorganisms (Dutaur and Verchot, 2007; Tate, 2015)(Figure I. 3). N_2O emissions from soil are driven by microbial nitrification (oxidation of NH_4^+ to NO_3^-) and denitrification (reduction of NO_3^- to N_2O) (Figure I. 3) (Bai *et al.*, 2014; Zhong *et al.*, 2014; Ortiz-Gonzalo *et al.*, 2018).

Production of greenhouse gases by soil microorganisms and roots can change rapidly temporally and spatially, which makes modeling efforts challenging (Drewitt *et al.*, 2005; Courtois *et al.*, 2018). While most studies dealing with soil greenhouse gas effluxes addressed the spatial and temporal variations, the vertical distribution of soil respiration is still little documented in very deep soils (Drewitt *et al.*, 2005). Trees with roots at depths of more than 10 m are common in tropical forests (Nepstad *et al.*, 1994; Saleska *et al.*, 2007; Broedel *et al.*, 2017). In a Brazilian Amazon forest, the consumption of CH_4 occurred mainly in the upper 10 cm of the soil profile, the production of N_2O mainly in the 0-25 cm soil layer whereas CO_2 was produced down to at least a soil depth of 11 m (Davidson *et al.*, 2004). In another Amazonian forest, CO_2 production in the 0.5-3 m soil layer accounted for 17 % of the total soil CO_2

production (Sotta *et al.*, 2007). Even if microbial activity is low in deep soil layers (Fontaine *et al.*, 2007), the huge soil volume explored by fine roots might lead to significant CO_2, N_2O and CH_4 productions within the entire rooting soil profile.

Measurements of GHG emissions at the soil surface provide an integrative estimate of the net production in the soil, but information on the patterns of production, consumption and transport of CO_2, CH_4 and N_2O within the soil profile remain poorly understood (Davidson *et al.*, 2006; Hashimoto *et al.*, 2007; Wiaux *et al.*, 2015). Understanding these patterns can provide insights into the possibilities of reducing GHG emissions. Data on the time course of GHG concentrations throughout soil profiles can provide additional information on heterogeneous gaseous exchange processes at depth that may be involved in the control of such surface fluxes. The comparison between the dynamics of CO_2 contents at various depths and the dynamics of production and mortality of fine roots throughout the soil profile observed with minirhizotrons could help us to better estimate and characterize the origin of the organic C emitted at the soil surface.

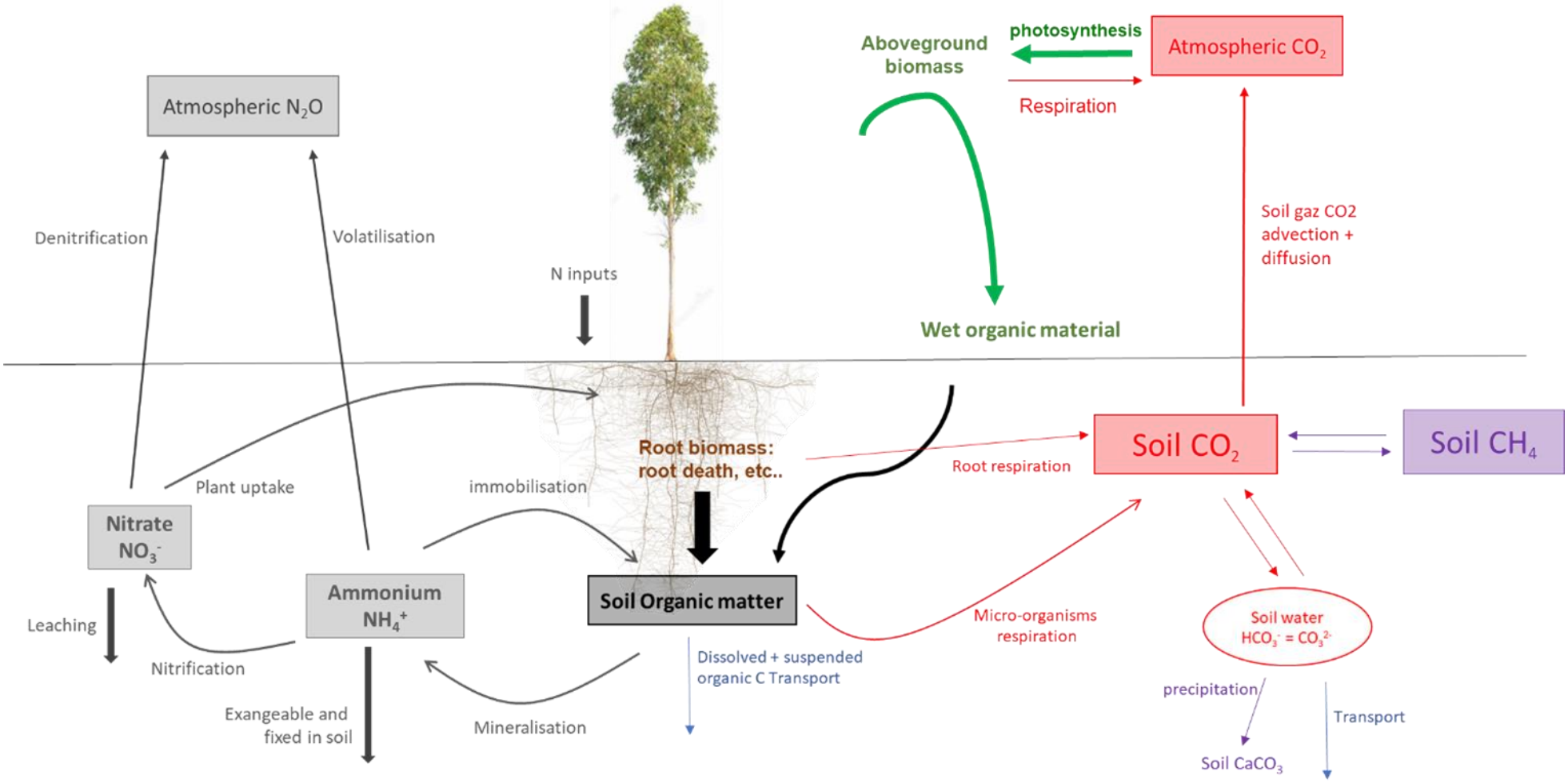

Figure I. 3: Schematic representation of belowground production, consumption and transport processes for *Eucalyptus* plantations.

I.8 Root dynamics in forest stands conducted in coppice

Coppice management could be an interesting option to face water deficit because the trees are likely to benefit from a root system already established preserving living biomass for the next generation and making it possible to access the deep soil layers where water availability can be higher than in the topsoil. Coppice-managed plantations, letting regenerate one or two shoots from the stump after the harvest, are common due to lower production costs, shorter cycle times and faster financial returns compared to replanting (Gonçalves *et al.,* 2014). The proportion of coppice-managed plantations has increased from 10 % of the total area of *Eucalyptus* plantations in 2008 to 25-35% in 2010. The effects of clear-cutting on fine root dynamics in coppiced-managed forest plantations are poorly documented and the trends are not clear. Sequential coring in Brazilian eucalypt plantations conducted in coppice showed that fine root density decreased in the 0-1 m soil layer the first 60 days after harvesting while fine root decomposition was accelerated (Mello *et al.*, 2007). Less intense root growth was observed in the first 10 cm of the soil at 90 days after cutting the trees. In addition, this study showed that the decomposition of fine roots was accelerated after the clear-cut (Mello *et al.*, 2007). An earlier study suggested that the growth of fine roots continues during the period after the harvest of trees in coppice-managed plantations of *E. camaldulensis* Dehn in Morocco; however, radial root growth was interrupted after cutting the trees (Riedacker, 1973). The most complete studies on root dynamics after regrowth have been carried out in coppice-managed *Populus* plantations. Dickmann *et al.* (1996) observed little changes in fine root production and mortality after clear-cutting down to a depth of 1 m. Berhongaray *et al.* (2015) showed that before cutting the trees, the mortality of fine roots was much lower than the production, while after the harvest, mortality exceeded the root production. However, these results were obtained only in the upper 80 cm of the soil, while studies carried out in eucalypt plantations in the state of São Paulo showed that approximately 50% of the roots of diameter <1 mm were located below 1 m (Laclau *et al.*, 2013). Cutting the trees leads to a significant production of organic matter via necromass and carbon compound exudates and therefore could be considerable at great depth (Werner and Schnyder, 2012; Berhongaray *et al.*, 2015) due to the amount of fine roots produced in these soil depths (Laclau *et al.*, 2013).

I.9 Root inputs and changes in soil organic matter dynamics in coppice-managed stands

Coppice management may influence carbon sequestration in the superficial soil layers in *Eucalyptus* plantations, but also in very deep soil horizons where the influence of fresh organic matter inputs on carbon storage is still poorly understood (Fontaine *et al.*, 2007; Derrien *et al.*, 2014). Removing the aboveground parts of trees can have a great impact on the ecosystem carbon balance and greenhouses gases emissions. Clear-cutting increases the solar radiation transmitted to the soil, raising the soil temperature and soil water contents through an interruption of stand transpiration (Londo *et al.*, 1999; Mello *et al.*, 2007). This may induce an increase in microbial activity, the decomposition of harvest residues left at the soil surface and enhance fine root mortality and decomposition (Parfitt *et al.*, 2001; Mello *et al.*, 2007). When decomposed, a part of the former tissues from the roots is incorporated into the soil organic matter (Strand *et al.*, 2008). Microorganisms are reactivated in response of the input of fresh organic matter. Specialized microorganisms develop rapidly and break down fresh organic matter. Their metabolites can activate a second class of specialized microorganisms, in dormancy, which can degrade the organic matter initially present and stored in the soil: this is the "priming effect" (Fontaine *et al.*, 2003). Kuzyakov *et al.* (2000) emphasizes the importance of characterizing microbial communities, their biomass but also their functioning (e.g. catabolic profile or enzymatic activity), and their structure (e.g. bacteria / fungi ratio) as a prerequisite for the evaluation of the underlying mechanisms. The consumption of soil organic matter by microbes results in the emission of CO_2 in aerobic conditions and CH_4 in anaerobic conditions. The “priming effect” could affect carbon storage in the soil and indirectly influence the nitrogen cycle. Indeed, CO_2 emissions in the soil profile influence the oxygen partial pressure, which is one of the factors controlling the transformation reactions of nitrogen substrates in the soil. Furthermore, the availability of carbon substrates is one of the main regulatory factors of the denitrification reaction (Groffman, 1991). Diffusion and transport of gases are inversely proportional to the water-filled pore space (WFPS%). Therefore, when the soil water content decreases, CO_2, CH_4 and N_2O reaches the surface quickly, the oxygen diffuses better throughout the soil profile, which influences important production and consumption processes (Heincke and Kaupenjohann, 1999; Clough *et al.*, 2005). Most of the studies dealing with soil carbon storage and greenhouse gas concentrations are limited to the upper meter of the soil profile (Harper and Tibbett, 2013). The consequences of tree harvesting on root dynamics and greenhouse gases production and consumption over the first year of the rotation in coppice-

managed plantations ask specific questions never studied within the entire rooting profile in deep tropical soils.

A literature review pointed out the importance of fine roots in deep soil layers for the growth and survival of plants and their ability to adapt to biotic and abiotic stresses. The foraging strategy adopted by root systems is influenced by water availability, so plants tend to develop deeper root systems when they are subject to water stress (Christina *et al.*, 2011; Bristiel *et al.*, 2018). Coppice management could be an interesting option to face water deficit because the trees are likely to benefit from a deep root system already established before harvesting the previous stand to produce new shoots. Despite recent insights highlighting the importance of fine roots at great depth, current knowledge of their dynamics is still extremely limited. Furthermore, root mortality supply organic matter at different soil depths, which is likely to affect greenhouse gas production and consumption processes. Therefore, improving our understanding of the spatiotemporal dynamics of GHG concentrations throughout deep soil profiles is an important issue to assess the environmental impact of silvicultural practices in tropical planted forests in a context of climate change.

CHAPTER 2: RESEARCH QUESTION AND SPECIFIC OBJECTIVES

The evaluation of the contribution of deep fine roots to the spatiotemporal dynamics of GHG concentrations throughout deep soil profiles is a metrological challenge and, in fact, a major scientific lock. The general objective of the thesis is to gain insight into fine root production and mortality in deep soil profiles with a joint characterization of greenhouse gases consumption and production. These effects have been monitored before and after harvesting all the trees in a highly productive *Eucalyptus grandis* plantation managed in coppice after clear-cutting, under two contrasting water supply regimes in São Paulo state, Brazil.

The specific objectives of the thesis are:

- To gain insight into the influence of rainfall reduction on fine root production and mortality throughout the soil profile in coppice-managed plantations;
- To assess the effect of clear-cutting and coppice management on fine roots dynamics down to the water table, here located at a depth of 17 m;
- To determine the vertical profile of CO_2, CH_4, and N_2O production rates from the topsoil to a depth of 17 m under two contrasting rainfall regimes.

The results obtained in this thesis are presented as three scientific articles and the organization of the different parts are summarized in Figure II. 1:

- Chapter 4: « Deep fine root dynamics in forest ecosystems ». This review will be submitted to the Journal Forest Ecology and Management, after co-authors revision.
- Chapter 5: « Consequences of clear-cutting and drought on fine root dynamics down to 17 m in coppice-managed eucalypt plantations ». This article has been submitted to the journal Forest Ecology and Management and is currently under minor revision.
- Chapter 6: « Consequences of clear-cutting and drought on CO_2, CH_4 and N_2O productions throughout deep soil profiles in coppice-managed eucalypt plantations ». This article will be submitted to the journal Global Biochemical Cycles.

The Chapter 7: « Modeling CO_2 production and transport in a deep Ferralsol under eucalypt plantations subjected to contrasted rainfall regimes » will not be published in a scientific journal because the development initiated in this thesis could not lead to exploitable results.

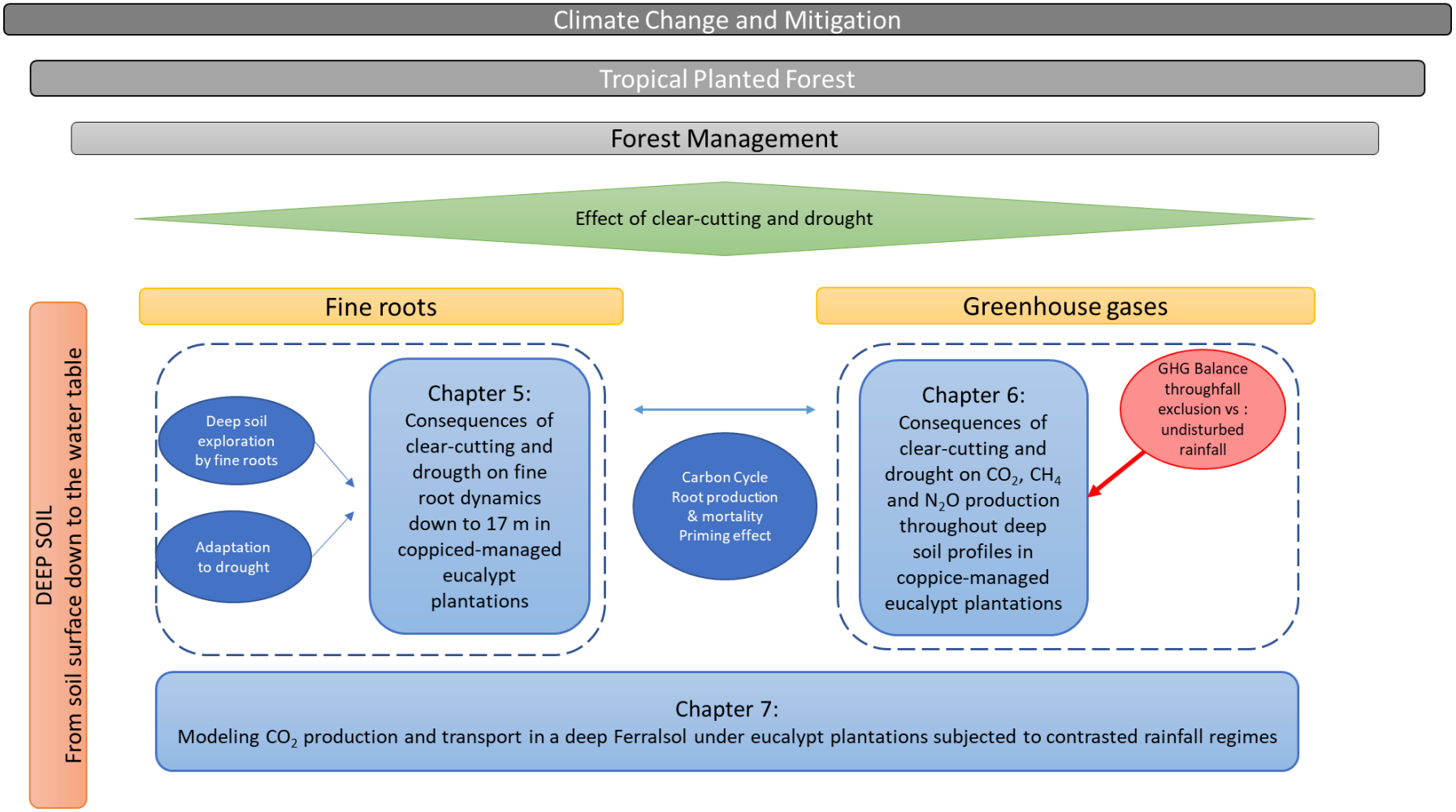

Figure II. 1: Conceptual diagram of the organization of the different parts of the thesis.

CHAPTER 3: STUDY SITE

To meet our objectives, we used a field trial in a clonal plantation of *Eucalyptus grandis* W. Hill ex Maiden located in the southeastern region of Brazil, in São Paulo state. Very simplified forest ecosystems such as monoclonal plantations offer interesting opportunities to understand more precisely some ecophysiological and biogeochemical processes impacting carbon and nutrient cycling. The studied field trial is particularly of interest to answer our questions because *Eucalyptus* trees exhibit a remarkable deep root system in homogeneous tropical soils, and they have the capability to be coppice-managed.

III.1 Characteristics of the experimental area

The work presented in this thesis was performed between 2015 and 2017 in Brazil at the Itatinga Forest Science Experimental Station owned by the Agricultural Superior School of Luiz de Quieroz (ESALQ) of São Paulo State University (USP). This experimental station, of an area of 2120 hectares, is located on the western plateau of São Paulo (23°02'S 48°38'W) at 300 km from the sea. This experimental site was heavily instrumented to quantify the dynamics of C, water and nutrient fluxes and to model the functioning of highly productive eucalypt plantations.

The climate of this region was humid subtropical with a dry winter (Cwa) according to the Köppen classification. Over the 15 years prior to this study, the mean annual rainfall was 1390 mm, the mean air temperature was 20°C and the mean relative humidity was 77%. Two seasons can be distinguished, a dry and cold season from June to September with a mean monthly temperature of 15°C, and a wet and hot season from October to May, with a mean monthly temperature of 25°C and heavy rainfalls (about 75% of the total annual rainfall).

The experiment was located on the upper part of a hill (slope <3%) at an altitude of 850 m. From 1945 to 1998, the area was planted with *Eucalyptus saligna* and since 1998 wooded with a highly productive plantation of *Eucalyptus grandis* W. Hill ex Maiden. *E. grandis* are native from the east coast of Australia, between latitudes 33°S (New South Wales) and 16°S (northern Queensland), in areas where precipitations are greater than 1000 m. In Brazil, the *Eucalyptus grandis* species is widely used due to the high quality of pulpwood, rapid growth and high productivity, and its suitability for coppice-management.

The soils were very deep (> 15 m) Ferralsols (IUSS Working Group WRB, 2015) developed on Cretaceous sandstone of medium texture. Clay contents were ranging from 160 mg g^{-1} in the

topsoil to about 250 mg g^{-1} at a depth of 15 m. Soil mineralogy is dominated by quartz, kaolinite and oxyhydroxides. Maquere (2008) characterized in detail the soil chemical attributes down to a soil depth of 6 m (Table III. 1).

Table III. 1: Chemical attributes of the soil of the experimental area down to a depth of 6 meters determined by Maquere (2008) (mean value of nine trenches).

Depth	ph		K	Ca	Mg	Na	H+Al	SB	CTC	V
m	water	KCl				$mmol_c\ kg^{-1}$				%
0-0.05	4.6	4.0	0.1246	0.2180	0.3115	0.0000	23.54	1.4	24.94	5
0.05-0.15	5.3	4.4	0.0467	0.0081	0.1303	0.0000	11.66	0.30	11.95	2
0.15-0.5	5.5	4.7	0.0286	0.0052	0.0316	0.0000	8.80	0.09	8.89	1
0.5-1.0	5.5	4.6	0.0161	0.0000	0.0124	0.0000	6.49	0.04	6.53	1
1.0-2.0	5.7	5.2	0.0305	0.0153	0.0153	0.0034	3.94	0.12	4.06	3
2.0-3.0	6.0	5.3	0.0133	0.0007	0.0103	0.0159	0.80	0.08	0.87	18
3.0-4.0	5.7	5.3	0.0393	0.0363	0.0375	0.0042	0.28	0.16	0.44	36
4.0-5.0	5.9	5.5	0.0298	0.0261	0.0253	0.0000	0.04	0.16	0.20	79
5.0-6.0	5.8	5.6	0.0287	0.0095	0.0164	0.0072	0.13	0.11	0.24	56

III.2 Experimental Layout

Changing water availabilities is an important issue in a context of global change as the continuing rise in atmospheric CO_2 levels may come along increased variability in rainfall distribution in many regions. A split-plot experimental design was set up in June 2010 with a *Eucalyptus grandis* clone used in commercial plantation by the Suzano Company (São Paulo, Brazil), to evaluate the impact of rainfall reduction and contrasting fertilizations on the productivity and adaptability of *Eucalyptus* plantations. The experimental layout contained six treatments (three types of nutrient supply x two water regimes) replicated in three blocks. The whole-plot factor was the water supply regime (‘‘exclusion” plots, -W, vs ‘‘non-exclusion” plots, +W) and the split-plot factor was the fertilization regime (Figure III. 1). In this thesis, we studied two treatments out of all those available in the experimental design: one treatment with undisturbed rainfall (+W) and one treatment with throughfall exclusion (-W). Treatments -W and +W were used to determine the water deficit effect (Figure III. 1).

The individual plots area was about 864 m^2. Trees were planted in June 2010 at a spacing of 3 m x 2 m with a stocking density of 1666 trees per hectare, e.g. 144 trees per plot totalizing 432 trees per treatment per 3 blocks. All the plots were fertilized at planting with 3.3 g P m^{-2}, 200 g

m^{-2} of dolomitic lime and trace elements, and at 3 months of age 12 g N m^{-2} and 0.45 mol K m^{-2} were applied as KCl. Field trials at the study site and in nearby areas on the same type of soil showed that the amounts of N, P, Ca, Mg and micronutrients applied in this experiment were not limiting for tree growth (Gonçalves *et al.*, 2007; Laclau *et al.*, 2009). Herbicides were applied to avoid the presence of understory species.

Since September 2010, throughfall was partially excluded in -W plots, using panels made of clear greenhouse plastic sheets mounted on wooden frames at heights ranging between 0.5 m and 1.6 m. Plastic sheets covered 37% of the area in the -W plots. A 50 cm deep trench was dug around the -W plots to limit the lateral development of *Eucalyptus* roots between + W and -W treatments. The dead leaves, branches and bark that fell on the plastic sheets were collected weekly and scattered under the gutters during the entire study. Water excluded from the gutters was collected weekly and samples were analyzed monthly to estimate the amounts of nutrients excluded during the study period. These amounts of dissolved nutrients in the water were replaced each year in each -W treatment (ammonium sulphate, KCl, NaCl, phosphorus ...) to distinguish the effect of water exclusion and nutrient deficiency.

Figure III. 1: Throughfall exclusion experiment located in the southeastern region of Brazil, at the Itatinga Forest Science Experimental Station in São Paulo state.

In +W and -W, the eucalypt stands were harvested six years after planting, in June 2016, and the plantation was coppice-managed thereafter. Several new shoots were regenerated from the stumps after the clear-cut and 1 or 2 shoots per stump were selected to maintain the same

stocking density and prevent the growth of additional new shoots. A third treatment served as a control, with undisturbed rainfall and no harvest (NH), to assess the clear-cutting effect.

Two deep permanent pits were excavated in +W and -W to gain access to the complete soil profile from the top soil down to the water table. A third pit was excavated in the non-harvested treatment (NH) down to 3.5 m to determine the clear-cut effect Figure III. 2).

Figure III. 2: Deep permanent pit down to a depth of 17 m in throughfall exclusion experiment at the Itatinga Forest Science Experimental Station in São Paulo state.

Root dynamics were studied using minirhizotron over 1 year before clear-cutting, then over 2 years in coppice, as well as in the non-harvested plot (NH) serving as a control, and fine roots (i.e. diameter < 2 mm) were sampled down to 17 m in +W, -W and NH. More complete descriptions of the methods used as well as the results obtained are presented in Chapter 5 « Consequences of clear-cutting and drought on fine root dynamics down to 17 m in coppice-managed eucalypt plantations »

CO_2, CH_4 and N_2O surface effluxes at soil surface were measured over three years using the closed-chamber method in -W, +W and NH. CO_2, CH_4 and N_2O concentrations in the soil were

measured from two permanent pits down to a depth of 15.5 m in -W and +W, as well as down to a depth of 4 m in NH. Measurements were carried out every 2 weeks over 3 months before clear-cutting, then over 19 months in coppice. More complete descriptions of the methods used as well as the results obtained are presented in Chapter 6 « Consequences of clear-cutting and drought on CO_2, CH_4 and N_2O productions throughout deep soil profiles in coppice-managed eucalypt plantations »

The chronology of the experiment is summarized in the following Figure III. 3.

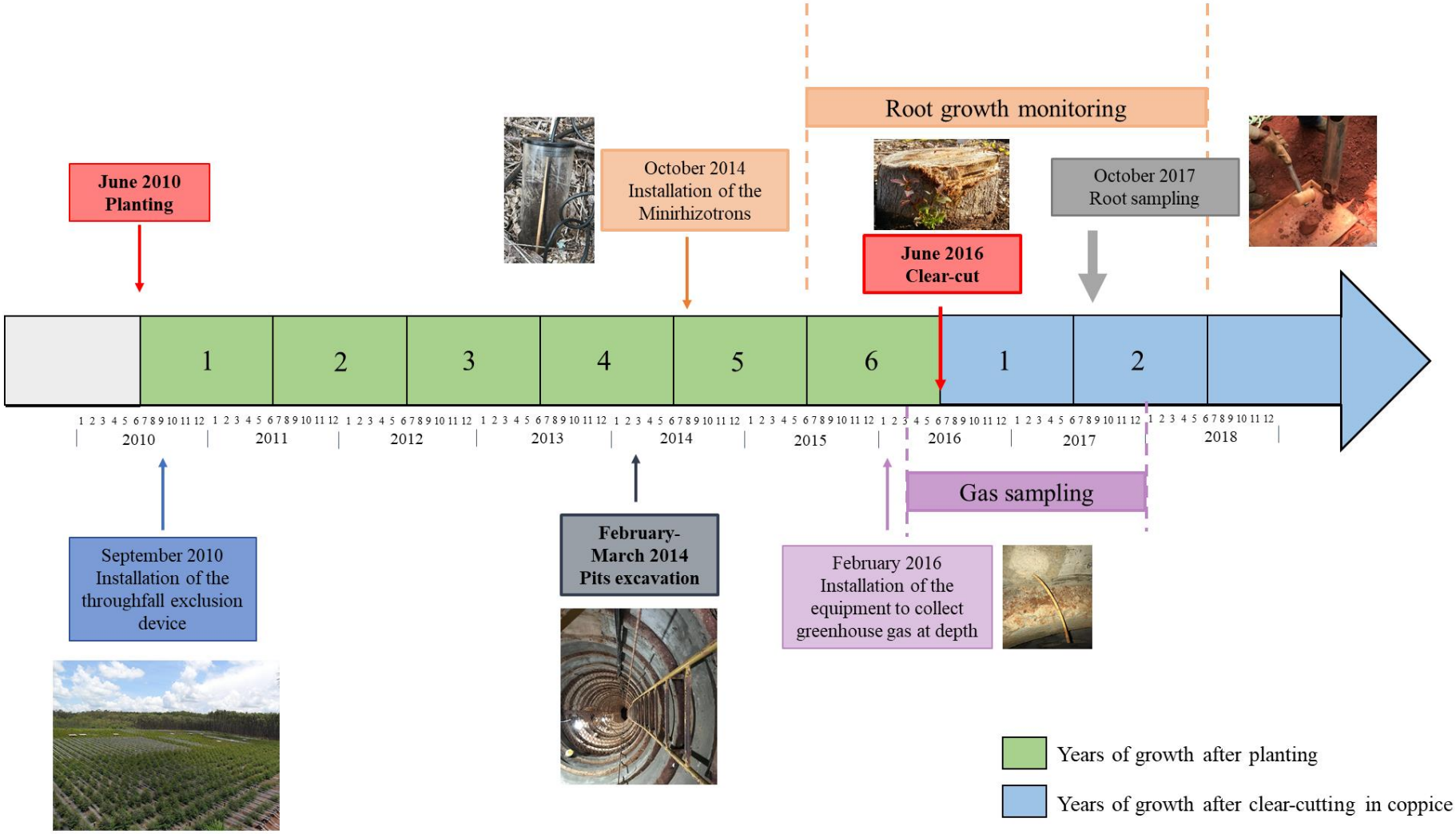

Figure III. 3: Chronology of the experiment

III.3 Summary of the previous works in this field trial (supplementary information)

Joint monitoring on this site of the main carbon fluxes (soil surface effluxes, aboveground and belowground biomass, litter fall, photosynthesis and leaf-level respiration, LAI), water (water balance in the soil, sap flow) and nutrients (accumulation in the trees and litter, dissolved fluxes in gravitational solutions) provided important advances in understanding the environmental control of biogeochemical cycles.

This experiment was highly instrumented with soil moisture measurements (down to 17 m depth), groundwater level (which varies around 17 m depth), soil temperature, growth radial (dendrometric ribbons), circumference and height, leaf potential, stomatal conductance, photosynthesis, leaf area index, trunk respiration, concentrations of sugars and minerals in the phloem, mineral concentrations at different depths in the soil solutions, litterfall, soil respiration, carbon allocation, root distribution down to the root front, root surfaces and sapwood surfaces…

Previous studies in this experimental set up studied the effect of K deficiency and/or Na deficiency and/or water deficit on cell and leaf expansion *i.e.* foliar concentration of nutrients, polyols and soluble sugars, leaf water parameters and leaf anatomy (Battie-Laclau *et al.*, 2013) stomata structural and physiological adjustments (Battie-Laclau *et al.*, 2014b), gross primary production, light use efficiency for carbon assimilation and absorbed radiation as the main parameters accounting for the changes (Christina *et al.*, 2015), photosynthetic physiology (Battie-Laclau *et al.*, 2014a), phloem transport of photosynthates (Epron *et al.*, 2015), wood formation (Ployet *et al.*, 2019), water use efficiency (Battie-Laclau *et al.*, 2016), tree water use and water seepage (Christina *et al.*, 2018), canopy conductance, sapwood area: leaf area ratio (Huber value) and sap flow driving force (Asensio *et al.*, 2019) and deep root functioning through rhizosphere versus bulk soil analysis (Pradier *et al.*, 2017). The incorporation of these results in process-based models, as well as further studies dealing with long-term effect of water availability on soil properties might contribute to evaluate adaptive changes induced by global changes in tropical *Eucalyptus* plantations.

K deficiency and water deficit affect traits involved in catching light resources, traits controlling CO_2 assimilation, traits related to transport via xylem and phloem and soil water and nutrient resources (Table III. 2). Indeed, K fertilization through drastic modifications in leaves physiology greatly increased gross primary productivity and above ground biomass production. A modeling approach revealed that soil water uptake parameters and leaf photosynthetic

parameters were the main drivers of gross primary productivity and light-use efficiency for carbon assimilation. Photosynthesis, anatomical and biochemical leaf treats in *E. grandis* had a functional relationship between K and Na supply. Osmotic adjustment, stomatal closure, higher phloem sugar concentration, higher velocity of C transfer in phloem and a decrease in leaf area index were observed when trees were subjected to water restriction and K deficiency. K fertilization and water availability modified xylem metabolome and transcriptome and impacts greatly wood structure, and notably vessel properties. Water deficiency also increased the mean soil depth of water withdrawal, decreased the residual soil water content in deep soil layers and lead to a drop in the water table.

Furthermore, reduced rainfall significantly increase exchangeable K and H_3O^+ within the rhizosphere and enhanced at great depth. A complementary study showed for the first-time ectomycorrhizal symbiosis in deep soil layers and the population of *Pisolithus sp.*, the dominated genus, was strongly dependent on soil depth (Robin *et al.*, 2019). Those results combined suggest a different root functioning in response to drought condition and especially in deep soil layers.

However, the influence of nutrient and water availability on root traits and root dynamics at depth remains poorly known despite its role in: i) microclimate, ii) tree growth during drought periods, iii) the potential for carbon sequestration in the deep layers of tropical soils, iv) the recycling of nutrients. Improving the understanding of the environmental control of rooting strategies in trees is of interest beyond the scope of *Eucalyptus* plantations in Brazil. Furthermore, the huge soil volume explored by fine roots might lead to significant CO_2, N_2O and CH_4 productions, and improving our understanding of the spatiotemporal dynamics of gas concentrations in deep soil layers is essential to quantify more accurately C source/sink fluxes in tropical soils. Comprehensive experimental studies are needed to improve the current biogeochemical models used to predict the effect of drought periods on greenhouse gas effluxes.

Table III. 2: Main results obtained in the experimental set up on the effects of drought, Na deficit and K deficit on soil parameters, tree biomass and anatomy, on the traits involved in the capture of light resources and/or controlling CO_2 assimilation and traits controlling the transport of water into the plant and transpiration. ↗ indicates a positive effect, ↘ indicates a negative trend and → indicates predominantly no effect.

	Na deficit	K deficit	Water deficit	References
Soil parameters				
Soil water content			↘	Battie-Laclau et al. 2014; Christina et al. 2015; Christina et al. 2018
Amount of water stored in ground		↗	↘	Christina et al. 2018
Deep seepage		↗	↘	Christina et al. 2018
H_3O^+ rhizo/ H_3O^+ bulk			↗	Pradier 2016
K_{ech} rhizo / K_{ech} bulk			↗	Pradier 2016
C rhizo/ C bulk			↗	Pradier 2016
H^+ Al^{3+} rhizo			↗	Pradier 2016
Biomass and Anatomy				
Tree height		↘	↘	Christina et al. 2015; Epron et al. 2015
Trunk circumference at breast height		↘	↘	Christina et al. 2015
Aboveground biomass	↘	↘	↘	Battie-Laclau et al. 2016; Battie-Laclau et al. 2014
Stem wood biomass	↘	↘	↘	Battie-Laclau et al. 2016
Wood production	↘	↘	↘	Battie-Laclau et al. 2016
Wood density		↘		Poyet 2017
Leaf area		↘	↘	Battie-Laclau et al. 2013; Battie-Laclau et al. 2014; Epron et al. 2015
Leaf thickness	↘	↘		Battie-Laclau et al. 2014
Leaf biomass		↘	↘	Epron et al. 2015
Chlorophyll content	↘	↘	↘	Battie-Laclau et al. 2014
Leaves palisade		↘		Battie-Laclau et al. 2014
Leaves spongy		↘		Battie-Laclau et al. 2014
Leaves intercellular space		↘		Battie-Laclau et al. 2014
Leaves paracytic stomata		↘		Battie-Laclau et al. 2014
Traits involved in the capture of light resources and/or controlling CO_2 assimilation				
LAI: Leaf area index	↘	↘	→	Battie-Laclau et al. 2014
Leaf water potential at predown	→	↘	↘	Battie-Laclau et al. 2014; Epron et al. 2015
Leaf water potential at midday	↘	↘		Battie-Laclau et al. 2014; Epron et al. 2015
Leaf gas exchange			↘	Battie-Laclau et al. 2014
Leaf net primary production	↘	↘	↘	Battie-Laclau et al. 2016
Aboveground net primary production	↘	↘	↘	Battie-Laclau et al. 2016
Stomatal conductance	↘	↘	↘	Battie-Laclau et al. 2016
Canopy conductance		↘	↘	
Crown CO_2 uptake		↘	→	Epron et al. 2015

Soil parameters	Na deficit	K deficit	Water deficit	References
Traits involved in the capture of light resources and/or controlling CO_2 assimilation				
Maximum rate of photosynthetic electron transport at 25°C		↘	↘	Christina et al. 2015
Maximum rate of rubisco carboxylase activity at 25°C		↘	↘	Christina et al. 2015
GPP: Gross primary production		↘	↘	Christina et al. 2015
LUE: Light use efficiency		↘	↘	Christina et al. 2015
aPar: absorbed photosynthetically active radiation		↘	↘	Christina et al. 2015
WUE: Water use efficiency		↘	→	Battie-Laclau et al. 2016
Traits controlling the transport of water into the plant and transpiration				
Sapwood cross section area		↘	→	Epron et al. 2015
Xylem sap flux		↘	→	Epron et al. 2015
Cambial activity		↘	↘	Poyet 2017
Canopy transpiration		↘	↘	Christina et al. 2018
Soil water stress index		↘	↗	Christina et al. 2018
Vessel diameter		↘	→	Poyet 2017
Hydraulic conductivity		↘	→	Poyet 2017
Traits involved in the capture of water and nutrient resources				
SRL			?	This thesis
SRA			?	This thesis
Root diameter			?	This thesis
Root biomass			?	This thesis
Root necromass			?	This thesis
Greenhouse gases (CO_2, N_2O and CH_4)				
Effluxes at soil surface			?	This thesis
Concentration in the soil			?	This thesis
Production within the soil profile			?	This thesis

CHAPTER 4: DEEP FINE ROOT DYNAMICS IN FOREST ECOSYSTEMS: WHY DIGGING DEEPER?

Abstract

Deep fine roots have been recently defined as fine roots encountered below a depth of 1 m and represent a large part of tree root system. While knowledge on deep root dynamics is constantly expending, our understanding of the phenology, morphology, anatomy and role of deep fine roots is still largely incomplete. In this review, the current knowledge about fine root production, mortality and longevity in deep soil layers, and the magnitude of the impacts and significance of deep fine root on carbon and nutrient cycling were discussed and highlighted. Deep fine roots are highly plastic in response to environmental conditions and soil resources. They present contrasting functional traits and functional specializations compared to shallow roots and are mainly oriented toward water acquisition and transport. Deep roots have a large impact on plant development and biogeochemical cycles through water and nutrient uptake, carbon allocation and sequestration in the soil. Therefore, modeling processes need to consider deep root functioning and dynamics accurately to better estimate net ecosystem productivity and nutrients, carbon and water cycles.

Key words: fine root growth, root traits, root longevity, root production, root mortality, carbon sequestration, tree plantations

IV.1 Introduction

Roots are plant's underground organs responsible for anchorage in the soil and absorption of water and nutrients needed for plant development. The anchorage function is provided by the largest roots and mainly involves physical interactions between roots and the soil. The absorptive function is more complex as it involves biogeochemical, biochemical and biological interactions between fine roots, soil, but also soil microorganism. Roots develop often symbiosis with soil bacteria and fungi. Fine roots have the ability to adapt to different environments to facilitate the development of the plant. Fine roots link plant metabolism to soil nutrient cycles, they are ephemeral and frequently replaced. They can modify the physical, chemical and biotic soil properties by their activities which can have repercussions not only at the plant scale but also at the ecosystem level (McCormack *et al.*, 2015). Indeed, fine roots play a major role in the global carbon (C) cycle, representing significant C input into the soil by the incorporation of exudates and root necromass, and generating a return of C to the atmosphere through respiration and decomposition processes (Balesdent and Balabane, 1996; Strand *et al.*, 2008). Root systems are known to be very heterogeneous and plastic, both architecturally and functionally, depending on plant age and its phenology, soil conditions and climate (Hodge *et al.*, 2009). Roots have the capacity to expand over large soil volume, particularly with shrubs and trees, and can be found at very great depths in xeric and drought ecosystems (Canadell *et al.*, 1996; Schenk and Jackson, 2002).

Fine root dynamics are defined as fine root production and mortality (Hendrick and Pregitzer, 1993; McCormack and Guo, 2014), finer root turnover and survivorship (Anderson *et al.*, 2003), senescence (Huck *et al.*, 1987) and fine root elongation rate (Germon *et al.*, 2016; Lambais *et al.*, 2017). Fine root dynamics are driven by both endogenous and exogenous factors like soil temperature and water content (Coll *et al.*, 2012), hormones (McAdam *et al.*, 2016), photosynthate and nutrient availability (Tierney and Fahey, 2002; Sloan *et al.*, 2016), which may be considerably heterogeneous along the soil profile (Figure IV. 1). However, deep fine root dynamics, functional significance and contribution to the water cycles and biogeochemistry processes at plant and ecosystem scale, are still poorly known. The objective of this study is to provide an up-to-date literature review on deep fine roots through their root traits, morphology and phenology, and through the biotic and abiotic interactions with the soil environment. Previous studies on deep fine root production, mortality and longevity, belowground C sequestration, symbiosis with mycorrhizae, and nutrient uptake will be reviewed more specifically.

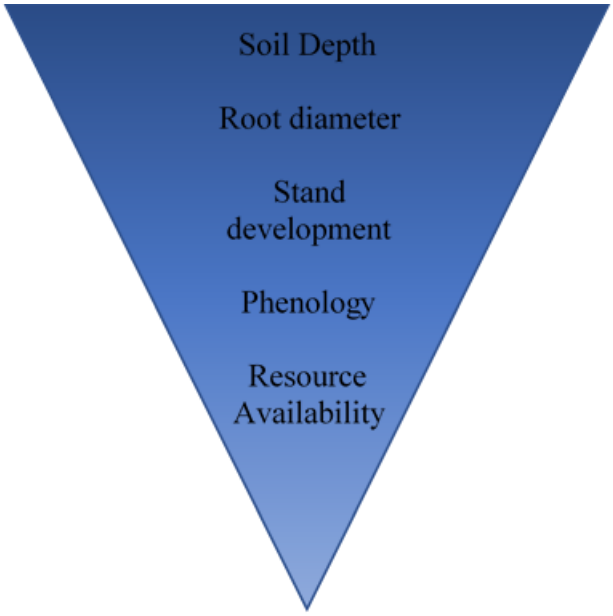

Figure IV. 1: Conceptual model illustrating the relative magnitude of different controls on fine-root dynamics (according to Coleman et al. 2018). The width of the pyramid and shading reflects the relative magnitude of each factor. As the width narrows and shading decreases, the magnitude of the response decreases.

IV.2 Defining deep fine roots

The importance of deep root system has been highlighted in several reviews (Canadell *et al.*, 1996; Maeght *et al.*, 2013; Pierret *et al.*, 2016) , but their dynamics and specificities are still poorly known. Numerous rooting depth characteristics have been examined since those studies, yet studies often claim that physiological and morphological characteristics of deep root systems are poorly understood. Recently defined by Maeght *et al.* (2013), deep roots are roots growing below a depth of 1 m. More and more studies highlight that rooting depth has been underestimated, particularly for shrubs and trees (Canadell *et al.*, 1996). Even if a small number of roots are found at great depth, deep roots likely have a key role in many processes of plant physiology, community ecology and geochemical cycles. Studies have demonstrated that deep roots are found in many biomes, where drought conditions are commonly exhibited (Nepstad *et al.*, 1994; Canadell *et al.*, 1996; Jackson *et al.*, 2000; Schenk and Jackson, 2002), but maximum rooting depth reported in the scientific literature is underestimated because few studies have focused on deeper horizons (Schenk and Jackson, 2002) and have artificially truncated the root distribution. Stone and Kalisz (1991) reported 37 examples of tree root colonization between 10 and 60 m below the soil surface, indicating that the maximum rooting depth varies considerably with climatic and soil conditions and between species. Canadell *et al.* (1996) reviewed that roots under different vegetation systems extend in deep horizons down to

9.5±2.4 m in deserts, 7.3±2.8 m in tropical evergreen forest and 15.0±5.4 m in tropical grassland and savannah, with maximum rooting depths of 68 m for *Eucalyptus* trees.

Once that considerable rooting depth has been highlighted, deep root distribution and dynamics need to be better described as it is still poorly studied compared to shallow root distribution. Indeed, deep root studies are scarce despite the recent technical advances and innovations because it is still difficult to access, time-consuming, expensive and requires a combination of several methods to obtain the most reliable results (Maeght *et al.*, 2013). Methods for monitoring deep roots are grouped into direct and indirect techniques depending on the equipment needed, research aim and their respective advantages and drawbacks (Vogt *et al.*, 1998; Hendricks *et al.*, 2006). N budget, ecosystem carbon balance, starch and carbon fluxes approaches have been used to study and quantify indirectly deep root functioning at considerable soil depths and are described in detail by Vogt *et al.* (1998). As for the direct methods, excavation, trenches, sequential coring, ingrowth cores, rhizotron, and minirhizotrons have been used to monitor root dynamics *in situ* and have been described in detail by Maeght *et al.* (2013). Both indirect and direct approaches present potential biases leading to overestimation or underestimation of root growth and decomposition. Despite all these methods, there is no scientific consensus on which the most effective and reliable method is determining the dynamics of fine roots. Understanding spatial and temporal fine root distribution and functioning in deep soil layers is crucial for a better understanding of biosphere-atmosphere interactions (Zeng *et al.*, 1998; Kleidon and Heimann, 2000), and hence for terrestrial biosphere models. Deep roots improve tree tolerance to drought (McDowell *et al.*, 2008; Nardini *et al.*, 2016) and store C in deep soil layers (Laclau *et al.*, 2013). Their importance in the C cycle and water flow has been demonstrated through modeling simulations (Saleska *et al.*, 2007; Christoffersen *et al.*, 2014). Therefore, to understand and predict in the proper way water cycles, C sequestration, and primary production and many others ecosystem services we should consider deep soil layers as dynamics and functional systems where fine roots may be different along the soil profile.

IV.3 Deep fine root morphology and architecture

Root morphology and architecture are important in determining a plant's availability to survive in stressful soil conditions and can influence considerably tree growth and ecosystem processes. Root system morphology and architecture vary largely between species but also within species (Cannon, 1949; Nibau *et al.*, 2008). Functional traits also vary with environmental conditions

and resource distribution (Ostonen *et al.*, 2007). Root diameter and root length are two of the main criteria taken into account to evaluate root function and behavior. Fine roots have been classified into distinct classes for different functions and metabolic activity: i) root for acquisition and uptake of soil resources, therefore classified as absorptive roots are extremely fine and are the most distal roots; ii) root for transport and storage of nutrients are larger roots higher up in the branching order, which are the main structural part of the fine root system. The high variability of the environmental conditions such as soil moisture and temperature or nutrient availability along the soil depth result in contrasting fine root functional traits among depths.

Deep fine root exploration also leads to an important metabolic cost for plants (Iversen, 2010) which affect changes in fine root traits (Prieto *et al.*, 2015). The specific root length (SRL) and specific root area (SRA) are the two main root traits affected by depth (Maurice *et al.*, 2010; Prieto *et al.*, 2015; Pinheiro *et al.*, 2016). They represent together the volume of the root in contact with the soil, and indirectly describe the capacity to take up limited soil resources by the plant (Maurice *et al.*, 2010). There are few studies on the variation of specific root length and specific root area according to soil depth (Makita *et al.*, 2011; Prieto *et al.*, 2015; Pinheiro *et al.*, 2016), but the trends are still not clear. Indeed, Pinheiro *et al.* (2016) found a slight increase in SRL and SRA with soil depth down to 13.5 m for 4 different genotypes of *Eucalyptus* in Brazil. Maurice *et al.* (2010) also found that SRL increased down to a depth of 3 m in *Eucalyptus* plantations of different ages. Adriano *et al.* (2017) found no significant changes in SRL with soil depth down to 8 m in *Citrus sinensis* plantations, but they observed a tendency toward an increase in mean fine root diameters. Gwenzi *et al.* (2011) found that roots tended to be thicker down to a depth of 1.4 m in *Acacia rostellifera* and *Melaleuca nesophila* plantations in Western Australia. Prieto *et al.* (2015) measured root functional traits in 20 plant communities in 3 climatic zones: tropical climate, montane climate, and sub-humid Mediterranean climate. They found that fine roots were thicker in deep soil layer down to a depth of 1.6 m compared to the shallow layers. On the contrary, Sochacki *et al.* (2017) observed that root diameter decreased systematically with depth down to 6 m in *Eucalyptus globulus* plantations in southwest Australia, and Bakker *et al.* (2009) found that SRL decreased with depth down to 1.2 m in *Pinus pinaster* plantations in the southwest of France. Fast-growing species require a rapid and efficient acquisition of above and belowground resources; therefore, they generally have a higher specific root area and specific root length compared to slow growing species particularly in deep soil layers (Ryser, 2006; Reich, 2014; Jo *et al.*, 2015).

Roots of high SRL and thinner diameter are often associated with a better exploitation capacity (Hodge, 2004), and they increase the amount of root surface area in contact with soil water. Establishing a deep root system with a high surface area allows the extraction of water from a larger soil volume (Jackson *et al.*, 2000). Deep roots are mainly oriented toward water acquisition and transport. Indeed, McElrone *et al.* (2004) found that deep roots of *Juniperus ashei*, *Bumelia lanuginosa*, *Quercus fusiformis* and *Quercus sinuate* had higher vessel diameter and larger tracheid than shallow roots in deep soil layers down to 18-20 m (Figure IV. 2). They also found that deep roots had a greater hydraulic conductivity. In line with those results, Pate *et al.* (1995) reported that roots at a depth of 2 m had larger xylem conduits, higher length, specific area, and hydraulic conductivities, up to 15 times higher than roots in shallow layers for Australian species. Physiological and anatomical adjustment of fine roots in deep soil seems to be developed by plants to maximize hydraulic efficiency. Despite those results, studies on fine root morphology and anatomy at great depth are still scarce.

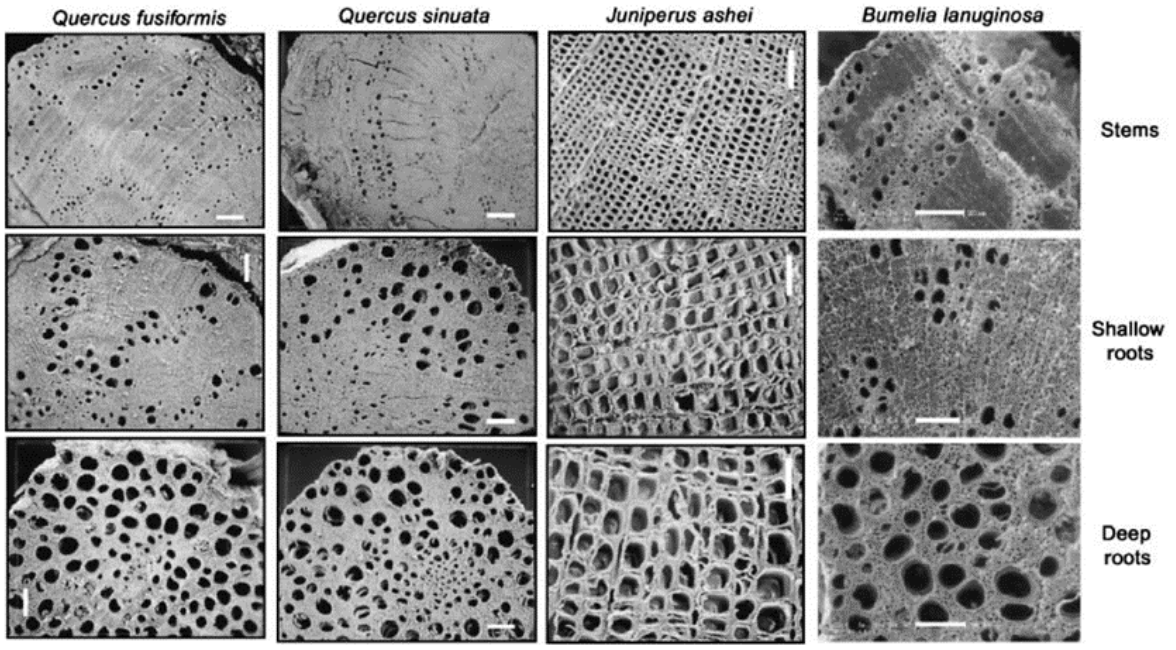

Figure IV. 2: Scanning electron micrographs of stems (top row), shallow roots (middle row) and deep roots (bottom row, 18-20 m) for *Quercus fusiformis*, *Juniperus ashei* Buchh., *Quercus sinuata* (Torr.) C.H. Mull and *Bumelia lanuginosa* tree species from the Edward's Plateau region of central Texas (According to McElrone et al. 2004).

IV.4 Deep fine roots phenology

Fine roots in deep soil layers present contrasting phenology compared to roots in shallow soil layers. Indeed, several studies have shown an asynchrony between shallow and deep root emergence. Maeght *et al.* (2015) observed that shallow fine root emergence of *Hevea brasiliensis* was synchronized with rainfall events and ceased during the dry season. On the contrary deep fine root emergence occurred at the end of the wet season and continued during the dry season below a depth of 2.5 m. Germon *et al.* (2016) also observed an asynchrony between shallow and deep roots in an agroforestry system in the south of France. They found that summer season was dominated by shallow root growth and winter season by deep root growth (> 2.5m). Similar results were observed in *Eucalyptus* plantations in Brazil, where root growth peaked below a depth of 3 m at the end of the dry season (Lambais *et al.*, 2017; Germon *et al.*, 2019). Germon *et al.* (2019) even found a negative correlation between the amount of extractable water in the topsoil and the flushes of growth in deep soil layers. Deep root growth seems to be related to overall tree water demand and controlled by the need for trees to use increasingly deeper water resources when water in the topsoil becomes scarce. Studies have shown that fine root elongation rates in deep soil are more intense than in the topsoil, reaching several centimeters per day (Laclau *et al.*, 2013; Germon *et al.*, 2016; Lambais *et al.*, 2017; Germon *et al.*, 2019). A near-symmetrical above and below ground growth rates were observed by Christina *et al.* (2011) in *Eucalyptus* plantations reaching 10.4 and 19.2 m in height and a maximum rooting depth of 9.2 and 15.8 m at ages of 1.5 and 3.5 years, respectively. Those results are in line with the growth rates of *Eucalyptus* observed by Lambais *et al.* (2017) in nutrient-poor soil of Brazil with a mean fine root elongation rate increasing with soil depth up to 3.6 cm day^{-1} below a depth of 3 m. Increasing root growth rate is often seen as another feature of drought adaptation (Annerose and Cornaire, 1994). This high root elongation rate in deep soil layers could be a response to improve water and nutrients uptake, which confirm the positive feedback from developing roots to develop shoot (Friend *et al.*, 1994). Fast root growth requires fast and efficient nutrient and water acquisition and thus a fast exploration of the deep soil layer. Environmental conditions and plant growth regulators may control those mechanisms (López-Bucio *et al.*, 2003). Deep soil layers are assumed to have higher soil water content and low-temperature variations compared to shallow layers (Du and Fang, 2014; Radville *et al.*, 2016). Thus, climatic factors being more buffered in the deep soil layer, fluctuating less during day and season, are not affecting equally root phenology along with the soil profile. Deep fine root phenology could be also controlled by other factors. Indeed, deep roots are more distant

from the shoot and the time to receive photosynthates would take longer to occur. A slow process phloem transport could also explain the delay between shallow and deep root growth currently observed. Yet there are still major uncertainties about the mechanisms controlling fine root phenology in deep soil layers.

IV.5 Mycorrhizal associations

Root systems are also characterized by their likelihood of association with mycorrhizal fungi. Interactions between plant root system and mycorrhizal fungi have many beneficial effects. It can provide a source of C for mycorrhizal respiration in exchange for increased exploitation and transfer of water and nutrient from soil to the plant. Soil depth influence the diversity of microorganism and community composition as nutrient supply and oxygen is less favorable for microbial decomposition (Eilers *et al.*, 2012; Santos *et al.*, 2016). However, an abundant and diverse microbial community and high level of bacterial biomass have been found in very deep soil layers (Dodds *et al.*, 1996; Gocke *et al.*, 2017; Zheng *et al.*, 2017). Stone *et al.* (2014) studied microbial community characteristics down to a depth of 1.4 m in a tropical soil of northeastern Puerto Rico. They unexpectedly found that extracellular microbial activity decreased in deep soil layers but C acquiring enzymes activities increased with depth. Recently, Robin *et al.* (2019) observed ectomycorrhizal roots down to a depth of 4 meters in a deeply weathered soil under *Eucalyptus* trees in Brazil. Deep root symbiosis with microorganism could potentially impact nutrient acquisition in deep soil layers and may enhance the exploitation of nutrient stock throughout the entire soil profile. Indeed, some studies highlighted that deep root and shallow roots had a different form of mycorrhizal symbioses (Rosling *et al.*, 2003; Clemmensen *et al.*, 2013). However, as for deep root functioning, studies on mycorrhizal symbiosis in deep soil are still very limited partly due to the difficulty to collect soil at such depths. Studying biological activities in deep soil layers is crucial for the scientific community and become increasingly important to address in future studies, yet it remains a methodological and technical challenge (Gocke *et al.*, 2017).

IV.6 Influence of soil resources and environmental parameters

Soil resources and environment parameters are constantly changing. Deep roots are highly plastic in response to heterogeneous resource distributions (Hodge *et al.*, 2009). They have the ability to adapt to changing resource availability and in particular to water availability, nutrients availability, soil texture, and atmospheric constituents. The variability of environmental factors

affecting root growth are buffered in deep soil condition compared to the considerable variability observed at the soil surface (Voroney, 2007). Differences in root structure, architecture, and production in deep soil layers might be attributed to differences in soil resources and physical properties.

Soil water content is maybe the main driver for root production in the deep soil layers. Adriano *et al.* (2017) observed that fine root mass of *Citrus sinensis* (L.) Osbeck increased in drought conditions, particularly in deep soil layers. Schenk and Jackson (2002) reported that rooting depths were correlated with the length of the dry season and annual precipitation in tropical climates. The longer dry season and lower annual precipitation, the deeper was the root system. Wang *et al.* (2015) also found a strong correlation between root mass and soil water content down to a depth of 21 m in planted grassland and shrubland in China. Drought can increase the proportion of fine roots in deep soil layers and/or the specific root area (Markesteijn and Poorter, 2009; Ma *et al.*, 2018; Zhou *et al.*, 2018). In a survey of 62 tropical tree species, Markesteijn and Poorter (2009) showed that trees increase the belowground biomass and the proportion of deep roots in response to dry conditions. Germon *et al.* (2019) showed an increase in SRL and SRA under drought conditions for *Eucalyptus* trees in Brazil. Root growth peaks have been shown in deep soil layers during dry periods for eucalypt and rubber trees in tropical soils (Maeght *et al.*, 2015; Lambais *et al.*, 2017). Plant species use a large range of rooting patterns to cope with periodic drought, from "drought tolerant strategies" with fine roots surviving in periodically dry soil to "drought avoiding strategies" shedding fine roots from dry soil horizons while rapidly developing roots in moister areas (Brunner *et al.*, 2015; Vries *et al.*, 2016; Bristiel *et al.*, 2018).

Root dynamics are also strongly correlated to the depth of the water table. Li *et al.* (2015) found a higher root growth rate when the water table was artificially deeper for *Alhagi sparsifolia* Shap. (Leguminosae). They found a root front growth of 0.66 cm d^{-1} when the water table was at a depth of 0.8 m and 1.5 cm d^{-1} when the water table was at a depth of 2.2 m. Canham *et al.* (2012) found a seasonal distribution of fine root biomass in deep soil layers following the fluctuations of the depth of the water table. A deeper water table level might induce a higher root elongation downwards (Stave *et al.*, 2005; Canham, 2011).

Soil texture also influences morphological acclimation of a deep root system. Root distribution has been showed to be shallower in sandy soils than in soil with a finer texture as shown in a tropical evergreen forest by Schenk and Jackson (2002). Xu and Li (2009) observed that root

of *Haloxyolon ammodendron* C.A. Mey penetrated down to a depth of 10 m in a sandy soil, while it only reached 3 m in a heavy soil. Roots had a higher root surface area in the sandy soil compared to roots growing in the heavy soil. Freycon *et al.* (2015) also compared root distribution down to 6 m for *Entandrophragma cylindricum* Sprague (Meliaceae) grown in two different soil types: Arenosols and Ferralsols in an African semi-deciduous rainforest. They found that fine root intersects on vertical pit walls were lower in coarse-textured soils (e.g. Arenosols) than in fine-textured soils (e.g. Ferralsols). However, Canadell *et al.* (1996) cited some examples of roots reaching great depths in rocky soils.

In most plant-soil systems, the amount of available nutrients is a growth-limiting factor. Indeed, McCulley *et al.* (2004) studied fine roots and nutrient distribution down to a depth of 10 m in five semi-arid and arid sites in the southwestern USA. They found that roots responded through morphological and physiological adjustment in response to nutrient availability within the soil profile. Bordron *et al.* (2019) found that fertilization increased the capacity of fine roots of *Eucalyptus grandis* to take up nutrients down to a depth of 3.2 m. They also found that adding nutrients increased root mass density, specific root length and specific root area along with the soil profile down to 3 m. On the contrary, Jourdan *et al.* (2008) showed that an increase in N content did not influence fine root biomass down to 3.0 m. Root growth and functioning are inhibited by high pH values due to nutrient deficiencies and specific ion toxicities (Hinsinger, 2001; Hinsinger *et al.*, 2003; Jacobs and Timmer, 2005). The response of fine root production and mortality on nutrient availability in deep soil layers is not completely understood and further studies are needed.

Many studies have focused on the impact of elevated CO_2 concentrations in the atmosphere on plant growth. Fine root responses in changing CO_2 conditions are still poorly known but some trends have emerged. Iversen (2010) reviewed that rising atmospheric CO_2 concentration tends to deeper rooting distributions of forested ecosystems. However, the set of studies used to focus on the upper soil profile down to usually a depth of 1 m. To our knowledge, only one study reached the threshold of 1 m depth concerning the influence of elevated atmospheric CO_2 on root properties. Indeed, Duursma *et al.* (2011) found that root water uptake of *Eucalyptus saligna* plantation in Australia was higher at depth > 1 m when trees are subjected to higher atmospheric CO_2 concentrations. Studies on the influence of elevated CO_2 on root biomass production and distribution need to be carried in much greater detail, and particularly in deep soil layers.

IV.7 Deep fine root biomass and carbon sequestration

It is essential to assess more precisely the capacity of ecosystems to store carbon to predict their potential role on the mitigation of the future economic and ecological challenges linked to climate changes. More and more research effort focused on C accounting across ecosystems. Fine roots play a major role in the global carbon cycle. They represent a significant C input to the soil by the incorporation of exudates and root necromass and generate a return of C to the atmosphere through respiration and decomposition processes (Balesdent and Balabane, 1996; Strand *et al.*, 2008). Estimating fine root production, biomass and turnover throughout the entire soil profile is therefore compulsory in this context.

Even if root density decrease with depth, some studies evidenced that total root biomass below the threshold of 1 m depth can account for more than currently admitted. Indeed, Maeght *et al.* (2015) found that more than 50% of the overall fine root biomass was below 1 m depth in a rubber plantation in Thailand. Fine root biomass between 1.0 and 4.0 m was 5.8 t ha^{-1} and was only 4.8 t ha^{-1} in the soil layer 0-1 m. In line with those results, Pinheiro *et al.* (2016) found that fine root length below 0.5 m represented 61-77% of the total fine root length down to the root front in *Eucalyptus* plantations. In an agroforestry system in the south of France, Cardinael *et al.* (2015) found that 35% of the total fine root intersection densities of walnut trees were below a depth of 2 m. In the same site, using the minirhizotron technique, Germon *et al.* (2016) measured that 25% of total fine root production was observed below a depth of 4 m. Falkiner *et al.* (2006) observed that *Eucalyptus grandis* root proliferation resulted in equal root length densities between 0-0.5 m and 2.4-2.8 m soil layers in another agroforestry system in south-eastern Australia. Germon *et al.* (2018) observed fine root biomass represented 31% of total biomass in *Eucalyptus grandis* monospecific stands, 52% in *Acacia mangium* monospecific stands and 37% in a mixture stands with 50% of both species in the 0-1 m soil layers. Furthermore, the proportion of roots at great depth increase with stand age: Ma *et al.* (2013) found that fine roots below 1 m represented 10.54 %, 29.62% and 42.42% of the total roots in 2 years, 4 years and 12 years of *Ziziphus jujube* Mill. CV. Lizao plantation in China, and Laclau *et al.* (2013) found that fine roots below a depth of 4 m represented 5%, 10% and 15% of total fine roots in year 1, 3.5 and 6 after planting of *Eucalyptus* plantation in Brazil. Li *et al.* (2019) observed that rooting depth increased with increasing stand age, reaching 23.2 m in a 22-year-old apple orchard stand in a sub-humid region of China, and roots below a depth of 1 m accounted for 49% of the total root biomass. Therefore, deep roots represent a high proportion

of the total root system and might contribute to sequestrating large amounts of C in soil (Matamala *et al.*, 2003; Rasse *et al.*, 2005) especially in deep soil layers (Kell, 2012).

Several studies have shown that root lifespan generally increases with soil depth (Hendrick and Pregitzer, 1996; Baddeley and Watson, 2005). Wells *et al.* (2002) found that root longevity of *Prunus persica* L. significantly increased with soil depth down to 1.6 m. Germon *et al.* (2016) also found a higher proportion of walnut root mortality in the topsoil that in the deep soil layers with a median life span of 167 days within the first soil meter and 208 days at 2.5-4.7 m depth in an agroforestry system in the south of France. But on the contrary, Maeght *et al.* (2015) found that root life span decreased with soil depth down to a depth of 4.5 m in a rubber tree plantation in Thailand. Maeght *et al.* (2015) found that roots between the depth of 1 and 2.5 m had a turnover of about 180-250 days and roots below 3 m of soil depth had a turnover of about 120 days. Lambais *et al.* (2017) found no significant differences in root lifespan of *Eucalyptus* trees between the topsoil and a depth of 6 m in Brazil, but root mortality represented almost 20% in the topsoil and only 3.4 % between 5 and 6 m depth. It is known that environmental parameters (e.g., temperature, water content, N availability, CO_2 and probably O_2 partial pressure) influence fine root turnover to variable degrees (Vogt et al. 1996), which could explain the opposite trends observed by those studies. Fine root decomposition rate affects the ability of ecosystems to store organic C and act as a sink for CO_2. The capacity of a different ecosystem to sequestrate carbon is related to the depth where roots are being decomposed. When roots are decomposed, parts of the former tissues are incorporated into the soil organic matter (Strand *et al.*, 2008). De Camargo *et al.* (1999) found that fine roots were being decomposed at least down to a depth of 5 m in eastern Amazon. Nepstad *et al.* (1994) estimated using isotopic tracers that C stocks below a depth of 1 m exceed organic soil C in the topsoil layer down to 1 m and the above-ground organic C in an Amazonian tropical forest. Indeed, the deep root system of this Amazonian forest input a large amount of organic carbon into the soil by root exudations, mycorrhizal associations and dead root tissues (Nepstad *et al.*, 1994). Moreover, the microbial biomass is lower in deep soil layers than in the topsoil, which in combination with oxygen limitations could enhance organic C sequestration as a result of low mineralization rates (Taylor *et al.*, 2002; Rumpel and Kögel-Knabner, 2011). Organic C derived from roots is generally more sequestered in the soil than organic C coming from the aboveground litter. The latter is more affected by physicochemical processes and has a different structural composition (Rasse *et al.*, 2005; Schmidt *et al.*, 2011; Menichetti *et al.*, 2015). It is therefore essential to estimate fine root production, biomass and turnover, and associated microbial communities throughout

the entire soil profile according to its environment to estimate adequately the overall C sequestration.

IV.8 Deep fine root functioning and nutrient mobilization

In addition to their importance for water uptake, deep fine roots may be essential to supply trees' nutrient requirements. Yet deep root functioning may vary over soil depth and the presence of roots in a given soil layer may not always correspond to root activity (Phillips *et al.*, 2016). Indeed, Göransson *et al.* (2006) showed that equal root abundance does not mean equal uptake of nutrients in *Quercus robur* L. plantations and that there is a contrasting potential uptake between deep and shallow layers. The depth of nutrient uptake performed by tree roots is commonly estimated using tracers. Bordron *et al.* (2019) showed a functional specialization of *Eucalyptus* fine roots to take up cations varying over depth from $^{15}NO_3^-$, Rb^+ (K^+ analog) and Sr^{2+} (Ca^{2+} analog) tracers injected at depths of 10, 50, 150 and 300 cm in a sandy Ferralsol soil in Brazil. They demonstrated that potential uptake of fine roots of 2-year-old *Eucalyptus* trees was significantly higher at a depth of 3 m than in the topsoil for Rb^+ and Sr^{2+}. Da Silva *et al.* (2011) also found an increase of the uptake rates at depth for K^+ and Ca^{2+} analogs compared to the topsoil in a 6-year-old *Eucalyptus* plantation, and on the contrary, they found that NO_3^- uptake was higher in the topsoil than at 3 m. Therefore, trees show great flexibility in root development and on their capability to adjust resource uptake in layers where the resources are available (Jackson *et al.*, 1990; Hutchings and de Kroon, 1994; Robinson, 1996). The functional specialization of deep roots is important to reduced nutrient losses through deep drainage (Laclau *et al.*, 2010). The previous estimations of nutrient leaching may therefore be over-estimated, as they were not considering nutrient uptake by deep roots when measuring only soil water concentration and fluxes at depth.

Deep roots strongly influence the water cycles and are essential to sustain high tree transpiration in the dry season (Kleidon and Heimann, 2000; Saleska *et al.*, 2007). Soil water content monitoring and the use of hydrogen and oxygen isotopes allow to estimate at which depth root water uptake is performed (Guderle and Hildebrandt, 2015; Beyer *et al.*, 2016; Koeniger *et al.*, 2016; Trogisch *et al.*, 2016; Beyer *et al.*, 2018). Stahl *et al.* (2013) showed that 46.1% of the trees were extracting water at or below a depth of 1.2 m in a tropical rainforest. Trees developed efficient strategies for water resource acquisition in deep soil layers using deep roots, which may also help sustain shallow root functioning during drought through hydraulic redistribution (Burgess *et al.*, 1998; Domec *et al.*, 2004; Bleby *et al.*, 2010). Burgess *et al.* (2001) have

demonstrated that *Eucalyptus* species have the capacity to redistribute water from wetter deep layers into shallow soil layers through roots. Furthermore, a modeling approach showed that the occurrence of rainfall events, changing from a week to another, had a strong impact on which depth water uptake occurs (Christina *et al.*, 2017). Recently, Li *et al.* (2019) showed that the extraction of deep soil water by the root system of apple orchard stands was mainly occurring at depths below 12 m in a sub-humid region of China. They also found that roots in the 1-12 m layer remained alive mainly to transport the water absorbed below 12 m as they might not extract additional water (Li *et al.*, 2019). Therefore, modeling processes need to consider deep root functioning to estimate net ecosystem productivity as a deep-water loss might contribute equivalently to the evaporation at the global scale than sallow soil water loss.

IV.9 Deep fine root and modeling approaches

Modeling architecture development and dynamics of the deep fine roots is hindered by the limited data available in the literature, yet indispensable to parametrize and evaluate the models. Root number, root diameter, root volume, and root radial distances are the main parameters of root system biomechanics (Nicoll and Ray, 1996; Danquechin Dorval *et al.*, 2016), varying considerably with soil layers. Soil depth strongly influences the root system architecture due to the heterogeneity of its environment. Root architectural models could simulate accurately spatial and temporal dynamics through a simulation of the root system in 3D. Integrating a vertically discretized soil into a model is particularly important as shallow and deep roots present different spatial and temporal dynamics. Model parametrization needs to consider shallow and deeper-rooted systems behaving differently according to the environmental factors and soil conditions. Indeed, these models are parametrized according to the definition of root types (e.g. RootTyp from Pagès *et al.* (2004) or DigR from Barczi *et al.* (2018)) or by branching order (e.g. OpenSimRoot from Postma *et al.* (2017)) or through parametric L-system modeling (Leitner *et al.*, 2010) which also might vary with soil layer. Such a model without discretized processes by soil layers is not capable to simulate accurately the impact of environmental conditions and soil resources on root development. Models devoted to simulating water and nutrient uptake dynamics need to integrate the functional specialization of deep roots and root growth plasticity in response to environmental conditions, in particular in deep soil layers. However, most root functioning rules are still not completely understood, especially for the ones occurring at great depth due to the extreme difficulty of measuring those processes in situ. Therefore, model calibration must cope with this knowledge and experimental limits. Architectural models can also be coupled with models simulating nutrient uptake (Mayer, 1999;

Bonneu *et al.*, 2012; Mayer *et al.*, 2012), water transport (Doussan *et al.*, 1998) or rhizospheric processes (Kim and Silk, 1999) or even reactive transport model (Mayer, 1999; Mayer *et al.*, 2012; Gérard *et al.*, 2017). Nevertheless, it can be convenient to simulate accurately the overall architecture and any specialized functions of deep roots compared to shallow roots.

Furthermore, deep roots have a strong influence on deep carbon stocks, which represent a significant amount of carbon (Jobbágy and Jackson, 2004) and can be partially renewed at the 10-year scale (Baisden and Parfitt, 2007; Koarashi *et al.*, 2012). Models predicting soil organic carbon stocks through a single soil layer modeling approach (Hansen *et al.*, 1991; Sallih and Pansu, 1993; Petersen *et al.*, 2005; Pansu *et al.*, 2010; Oelbermann and Voroney, 2011) are therefore not considering deep soil and their roots that play an active role in carbon cycling. In recent years, an awareness of the importance of deep carbon stocks by the modeling community has led to the development of different discretized models depending on the soil depth (Braakhekke *et al.*, 2011; Braakhekke *et al.*, 2013; Guenet *et al.*, 2013; Taghizadeh-Toosi *et al.*, 2014). However, modeling accurately deep root dynamics and their active role for carbon allocation, nutrient and water uptake according to the plant development over time remains a major challenge for the next decades.

Conclusion

Deep root systems are complex components of ecosystems. Deep roots display different functional specialization, anatomy and morphology and are mainly oriented toward water acquisition and transport (Table IV. 1). Climatic factors affect differently root phenology along with the soil profile as there are more buffered in deep soil layers. Research devoted to deep fine root dynamics linking anatomical, architectural and functional characteristics along very deep soil profiles to site characteristics, species and soil conditions should be carried out to gain insight into their impact on C, water and nutrient cycles. Deep fine root display plasticity by responding to changing soil conditions and have an important role for ecosystem functioning. It is then crucial to focus the research efforts on the understanding of deep root dynamics and associated functions which could have a significant influence on climate change mitigation.

Table IV. 1: Root traits at depth compared to the top soil. ↗ indicates a predominantly positive trend, ↘ indicates a predominantly negative trend and → indicates predominantly no effect. Categories of root traits and individual root traits are adapted from Brunner et al. 2015, Brunner et al. 2009, McCormack et al. 2012, Prieto et al. 2015.

Root traits categories	Root trait	At depth	References
Growth	Root biomass		Markesteijn and Poorter 2009; Jackson et al. 1996; Gwenzi et al. 2011; Ma et al. 2013
	Root density		Freycon et al. 2015; Gwenzi et al. 2011 ; Ma et al. 2013
	Lifespan		Germon et al. 2016; Hendrick and Pregitzer, 1993; Eissenstat and Yanai, 1997
	Mortality		Lambais et al. 2017; Germon et al. 2016
	Root elongation rates		Germon et al. 2019; Lambais et al. 2017 ; Germon et al. 2015
Architectural	Branching		David et al. 2013
	Root area index		Pinheiro et al. 2016; Germon et al. 2017
	Root tissue density		Bordron et al. 2019
	Specific root length		Maurice et al. 2010; Pinheiro et al. 2016 ; Prieto et al. 2015
	Specific root area		Pinheiro et al. 2016
	Diameter		Adriano et al. 2016; Prieto et al. 2015
Anatomical	Conduits		McElrone et al. 2004
	Mean vessel diameter		David et al. 2013; McElrone et al. 2004
Biotic	Mycorrhizas		Robin et al. 2019
Chemical	Root Carbon (C) concentration		Prieto et al. 2015
	Root Nitrogen (N) concentration		Prieto et al. 2015
	Soluble concentration		Prieto et al. 2015
Biochemical	Cellulose		Prieto et al. 2015
	Lignin		Prieto et al. 2015
Physiological	Sap velocity		Jonhson et al. 2014
	Hydraulic conductivity		McElrone et al. 2004; McElrone et al. 2007 Jonhson et al. 2014
	Specific relative uptake potential of Rb^{+}		Da Silva et al. 2011; Bordron et al. 2018
	Specific relative uptake potential of SR^{2+}		Da Silva et al. 2011; Bordron et al. 2018
	Specific relative uptake of NO_3^{-}		Da Silva et al. 2011; Bordron et al. 2018

Appendix IV. 1: Images of fine roots of *Eucalyptus* trees scanned in minirhizotron installed in the deep permanent pits in Brazil in the topsoil (top) and at great depth (bottom).

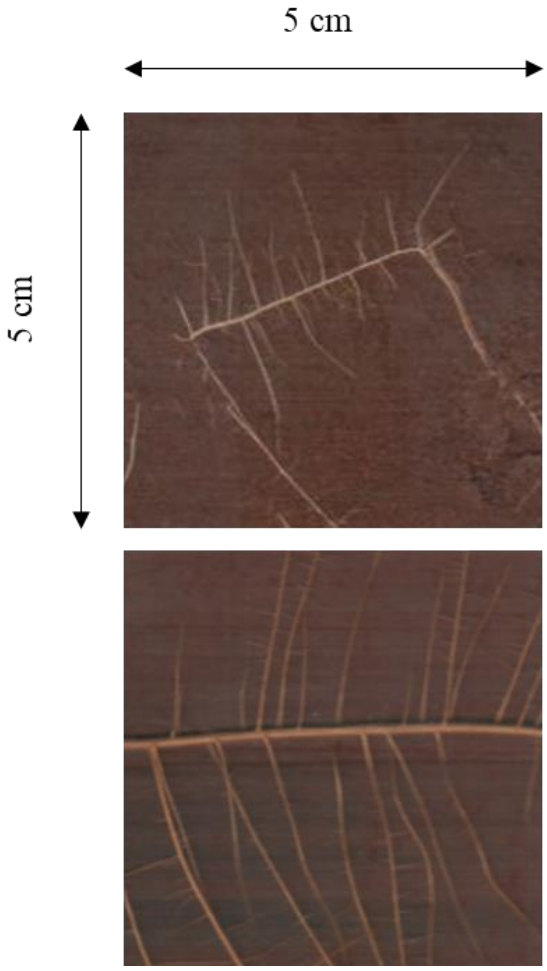

Image at a depth of 0.5 cm

Image at a depth of 11.5 m

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CHAPTER 5: CONSEQUENCES OF CLEAR-CUTTING AND DROUGHT ON FINE ROOT DYNAMICS DOWN TO 17 M IN COPPICE-MANAGED EUCALYPT PLANTATION

We saw in chapter 4 that despite the actual insights about the importance of fine roots in depth, current knowledge of their dynamics is still extremely limited. The evaluation of the contribution of fine roots in deep soil layers is a real metrological challenge and, in fact, a major scientific lock, which is important to raise to study the sustainability of agroecosystems and forest plantation, as well as their resilience, in the context of global changes.

The central objective of this following chapter is to assess the effect of clear-cutting and drought on fine-root production throughout deep soil profiles down to the water table in coppice-managed Brazilian eucalypt plantations. These effects were monitored before and after trees harvest in a coppice-managed *Eucalyptus grandis* plantation under two contrasting water supply regimes.

This chapter presents the results of 3 years of root dynamics monitoring and was submitted to the journal Forest Ecology and Management as scientific article with the title: “Consequences of clear-cutting and drought on fine root dynamics down to 17 m in coppice-managed eucalypt plantations”. Co-authors associated with this project are Christophe Jourdan, Bruno Bordron, Agnès Robin, Yann Nouvellon, Lydie Chapuis-Lardy, José Leonardo de Moraes Gonçalves, Céline Pradier, Iraê Amaral Guerrini and Jean-Paul Laclau.

After a careful revision by the editors and reviewers this paper is pending in “minor revision”. The co-authors and I are currently answering to the reviewer’s comments and suggestion, and a resubmission is scheduled for beginning of May.

Consequences of clear-cutting and drought on fine root dynamics down to 17 m in coppice-managed eucalypt plantations

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Abstract

Improving our understanding of the spatiotemporal dynamics of fine roots in deep soil layers is of utter importance to manage tropical planted forests in a context of climate change. Our study aimed to assess the effect of clear-cutting and drought on fine-root dynamics down to the water table in Brazilian ferralsol under eucalypt plantations conducted in coppice. Fine roots (i.e. diameter < 2 mm) were sampled down to 17 m deep in a throughfall exclusion experiment comparing stands with 37% of throughfall excluded by plastic sheets (-W) and stands without rain exclusion (+W). Root dynamics were studied using minirhizotron in two permanent pits down to 17 m deep, over 1 year before clear-cutting, then over 2 years in coppice, as well as down to 4 m deep in a non-harvested plot (NH) serving as a control. After harvesting, a spectacular fine root growth of trees conducted in coppice occurred in very deep soil layers (> 13 m) and, surprisingly, root mortality remained extremely low whatever the depth and the treatment. Total fine-root biomass in coppice down to 17 m depth was 1266 and 1017 g m^{-2} in +W and -W, respectively, at 1.5 year after the clear-cut and was 1078 g m^{-2} in NH 7.5 years after planting. Specific root length and specific root area were about 15% higher in -W than in +W. Proliferation of fine roots at great depths could be an adaptive mechanism for tree survival, enhancing the access to water stored in the subsoil. The root system established before clear-cutting provides access to water stored in very deep layers that probably contribute to mitigate the risk of tree mortality during prolonged drought periods when the eucalypt plantations is conducted in coppice after the clear-cut.

Key words: Brazil, coppice, deep ferralsol profile, deep root growth, *Eucalyptus grandis*, minirhizotron, throughfall exclusion

V.1 Introduction

Future climate projections predict longer and more severe dry periods in tropical and subtropical regions (Dai, 2011; He & Soden, 2017; Solomon et al., 2009). Extensive tree mortality triggered by drought and changes in rainfall patterns has been reported worldwide (Allen, 2009; McDowell et al., 2018; Williams et al., 2013). Tree survival greatly depends on rooting system behavior and functioning (Christina et al., 2017b; Markewitz et al., 2010; Nepstad et al., 1994; Pierik & Testerink, 2014), as plant growth is highly dependent on the absorptive function of fine roots for water and nutrients (Hinsinger, 2001). Fine roots also play a major role in the global carbon (C) cycle, representing significant C input into the soil by the incorporation of exudates and root necromass, and also generating a return of C to the atmosphere through respiration and decomposition processes (Balesdent & Balabane, 1996; Strand et al., 2008). Improving our understanding of how root systems respond to drought is therefore crucial for terrestrial biosphere models to predict the effect of climate change on tree survival and carbon sequestration in forest and tree-based ecosystems.

Plant species use a large range of rooting patterns to cope with periodic drought, from "drought tolerant strategies" with fine roots surviving in periodically dry soil, to "drought avoiding strategies" shedding fine roots from dry soil horizons while rapidly developing roots in moister areas (Bristiel et al., 2018; Brunner et al., 2015; Vries et al., 2016). Drought can increase the root-to-shoot ratio, the root area or root length-to-leaf area ratio, as well as the proportion of fine roots in deep soil layers and/or the specific root area (Ma et al., 2018; Markesteijn & Poorter, 2009; Zhou et al., 2018). Root growth peaks have been shown in deep soil layers during dry periods for eucalypt and rubber trees in tropical soils (Lambais et al., 2017; Maeght et al., 2015a). Drought tolerance strategies are common for herbaceous plants, while drought avoiding strategies are often adopted by trees in evergreen tropical forests (Brunner et al., 2015). Despite the crucial role of fine roots in coping with drought, root phenology remains poorly understood in comparison to leaf ecophysiology (Radville et al., 2016a).

Deep roots commonly reported as roots growing beyond 1 m in depth can play an important role in supplying water to trees (Kell, 2012; Pierret et al., 2016a). Trees can be deeply rooted (Canadell et al., 1996; Schenk & Jackson, 2002), and some studies suggest that very deep roots (at depths > 10 m) are common in highly weathered tropical soils (Broedel et al., 2017; Nepstad et al., 1994; Saleska et al., 2007). Even though low fine root densities are generally found at great depth (Pierret et al., 2016a), they can take up substantial amounts of water needed for tree

survival during drought periods (McDowell et al., 2008; Nardini et al., 2016). Deep roots can also contribute to closing the biogeochemical cycles in tropical forests through nutrient uptake in deep soil layers (Bordron et al., 2018; Da Silva et al., 2011; Jobbágy & Jackson, 2004; Lehmann, 2003), which reduces nutrient losses by deep leaching (Laclau et al., 2010; Lehmann & Schroth, 2003). While many studies show that the role of very deep roots in tropical forest functioning and productivity has been greatly underestimated (Freycon et al., 2015; Jackson et al., 2000; Markewitz et al., 2010), as far as we are aware, fine root dynamics and mortality have never been studied at depths > 10 m.

Eucalyptus plantations cover more than 20 million hectares and account for around 8% of forest plantations in the world (Booth, 2013). The diversity of *Eucalyptus* species planted in tropical regions has led to a wide range of products and management practices (Gonçalves et al., 2013). Although many *Eucalyptus* plantations are intensively managed to produce raw materials for industry (mainly pulp and paper, but also solid-wood products, fiberboards and charcoal for steel production), used as a domestic source of energy and also contributes to alleviating poverty in developing countries (Cossalter & Pye-Smith, 2003). Most eucalypt plantations are located in areas with low soil fertility and prolonged drought periods (Keenan et al., 2015). Coppice management could be an adaptive solution to cope with water deficit in these plantations, because the sprouts growing on stumps are likely to benefit from the pre-existing root system that explores deep soil layers where water availability is generally higher than in the topsoil. The effects of clear-cutting on fine root dynamics in coppiced-managed forest plantations are poorly documented and tendencies are not clear. Sequential coring in Brazilian coppiced-managed eucalypt plantations showed that fine root density decreased in the 0-1 m soil layer in the first 60 days after harvesting, while fine root decomposition was accelerated (Mello et al., 2007). Fine root mortality exceeded fine root production after clear-cutting in *Populus* stands (Berhongaray et al., 2015). In contrast, Dickmann et al. (1996) observed little change in fine root production and mortality in the 0-1 m soil layer after clear-cutting in other *Populus* stands. While 50% of fine root biomass can be found below a depth of 1 m in tropical eucalypt plantations (Christina et al., 2011b; Laclau et al., 2013a), fine root dynamics in coppice-managed forests have only been studied in the upper meter of the soil profile.

Our study was carried out in *Eucalyptus grandis* (Hill ex. Maid) stands planted in a throughfall exclusion experiment in Brazil (Battie-Laclau et al., 2014b). We aimed to gain insight into the effects of contrasting rainfall regimes on fine root dynamics in coppice-managed eucalypt plantations after tree clear-cutting in very deep tropical soils. We put forward the hypothesis

that trees invest in belowground biomass in response to throughfall exclusion, in order to increase the exploration of fine roots in deep soil layers (H1), and that they adjust fine root traits to increase the specific root area, in order to capture more resources per gram of C invested in fine roots (H2). In addition, we put forward the hypothesis that *Eucalyptus grandis* stands with clear-cutting and tree regrowth in coppices stimulate fine root growth in both shallow and deep soil layers (H3) and increase root mortality throughout the soil profile (H4).

V.2 Materials and Methods

V.2.1 Study site

The experiments were conducted at the research station owned by the Luiz de Queiroz College of Agriculture (ESALQ) near Itatinga (São Paulo, Brazil, 23°02′S 48°38′W). The study area, located 300 km from the sea, has a relief typical of the São Paulo Western Plateau, with a topography varying from flat to hilly (slopes < 3%). The altitude is 850 m, and the climate is humid subtropical with a dry winter (Cwa) according to the Köppen classification. Over the 15 years prior to this study, the mean annual rainfall was 1390 mm (with 74% between October and May), and the mean air temperature and relative humidity were 20°C and 77%, respectively. A dry and cool (15°C) season occurs between June and September. The total rainfall over the study period (from May 2015 to July 2018) was 5629 mm. The annual rainfall was 1,557 mm and 2,303 mm in 2016 and 2017, respectively. The soils are very deep Ferralsols (IUSS Working Group WRB, 2015) developed on Cretaceous sandstone. Clay content ranges from 160 mg g^{-1} soil in the topsoil to about 250 mg g^{-1} soil at a depth of 15 m, and clay minerals are mainly kaolinite (Christina et al., 2015).

V.2.2 Experimental layout

A split-plot experimental design was set up in June 2010 with a *Eucalyptus grandis* clone used in commercial plantations by the Suzano Company (São Paulo, Brazil). The experimental layout with 6 treatments and 3 blocks was described in detail by Battie-Laclau et al. (2014b). We studied two treatments out of all those available in the experimental design: one treatment with undisturbed rainfall (+W) and one treatment with throughfall exclusion (-W), which was equipped with plastic sheets installed since September 2010, allowing the exclusion of 37% of throughfall (Battie-Laclau et al., 2014b). Treatments -W and +W were used to determine the water deficit effect. The trees were planted in June 2010 at a spacing of 3 m x 2 m with a

stocking density of 1666 trees per hectare. The plots received a standard commercial fertilizer (at planting: 3.3 g P m^{-2}, 200 g m^{-2} of dolomitic lime and trace elements; at 3 months of age: 12 g N m^{-2}, 0.45 mol K m^{-2} applied as KCl) and herbicides were applied to avoid the presence of other understory species. In +W and -W, the eucalypt stands were harvested six years after planting, in June 2016, and the plantation was coppice-managed thereafter. Several new shoots were regenerated from the stumps after the clear-cut and 1 or 2 shoots per stump were selected to maintain the same stocking density and prevent the growth of additional new shoots. A third treatment served as a control, with undisturbed rainfall and no harvest (NH), to assess the clear-cutting effect. Tree height and circumference at breast height were recorded each year after the clear-cut on 36 central trees per plot during the first rotation (May 2010 - June 2016) and during the second rotation in coppice (starting in June 2016).

V.2.3 Soil water monitoring

The volumetric soil water content (SWC) was monitored in the +W and -W treatments throughout the study period at half-hourly intervals using CS616 probes (Campbell Scientific Inc., Logan, UT, USA) installed at the following depths: 0.15, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14 and 16 m, with 3 probes at each depth in block 1. Extractable water (mm) was calculated as the difference between the current soil water stock (mm) and the minimum soil water stock (i.e. lower limit of soil water content in mm) (Granier et al., 1999).

V.2.4 Deep permanent pits for root phenology observations

Between February and March 2014, two deep permanent pits were excavated in +W and -W in block 1 to gain access to the complete soil profile from the top soil down to the water table. The pits were 1.5 m in diameter and reached a depth of 17 m and were located between four *Eucalyptus* trees (Figure V. 1) at a distance of 90 cm, 90 cm, 130 cm and 130 cm from each four trees respectively. The pit walls were made of concrete rings in direct contact with the soil. Clear-colored roofs of the same diameter as the pits were used to prevent light and rain entering the pits. Platforms were set up at two-meter intervals in the pits, equipped with artificial lighting and fans used during working sessions, allowing access and safe work down to the water table (Figure V. 1). The high cost of opening up and securing these pits prevented further replications in the other two blocks. However, other measurements, including deep fine root sampling and tree growth were carried out in all three blocks, making it possible to extrapolate some of the

observations made in the two deep pits. In October 2015, a third pit was excavated in the non-harvested treatment (NH) down to 3.5 m to determine the clear-cut effect.

V.2.5 Minirhizotrons

Fine root dynamics were studied through transparent polyvinyl chloride tubes (length:180 cm; inner diameter: 6.5 cm), commonly called minirhizotrons (Maeght et al., 2013). In October 2014, twenty-four transparent minirhizotrons were installed using a powerhead drill in the +W and -W treatments of block 1. Outside the pits, three minirhizotrons were inserted into the soil surface in the same plots a minimum of 10 m apart and at a distance of 90 cm from the trunk (Figure V. 1). Nine tubes were inserted into the pit walls down to a depth of 17 m: two tubes at a depth of 1 m and one tube at depths of 3.5, 5.5, 7.5, 9.5, 11.5, 13.5, 15.5 m (Figure V. 1). The tubes were inserted at a 45-degree angle and allowed an observation depth of 1.3 m below the depth at which they were inserted into the soil profile. In October 2015, 3 additional tubes were installed at the soil surface in the +W and -W treatments of blocks 2 and 3 (12 tubes in total) to increase the number of replications in the top meter. In October 2015, seven tubes were installed in the NH treatments (3 into the soil surface, 2 tubes inserted at depths of 1 m and 3.5 m into the pit walls).

Root dynamics were recorded using a circular scanner system (CI-600 Root Growth Monitoring System, CID, USA). This scanner was selected for the quality of the images it produces, an essential element for the analysis (Graefe et al., 2008). In order to have a significant stabilization period after soil disturbance from the installation, fine root monitoring began eight months after minirhizotron installation (Germon et al., 2016; Graefe et al., 2008). Eight images (21.59 x 19.56 cm, 100 dpi) per tube (43 tubes in total) were taken every two weeks for more than three consecutive years from May 2015 to July 2018: over one year before the clear-cut and two years after harvesting, in a coppice. Images were taken at a resolution of 100 dpi, as we obtained the same root lengths and diameter compared to images of 300 dpi and 600 dpi, and it was less time-consuming in the field than with a higher resolution.

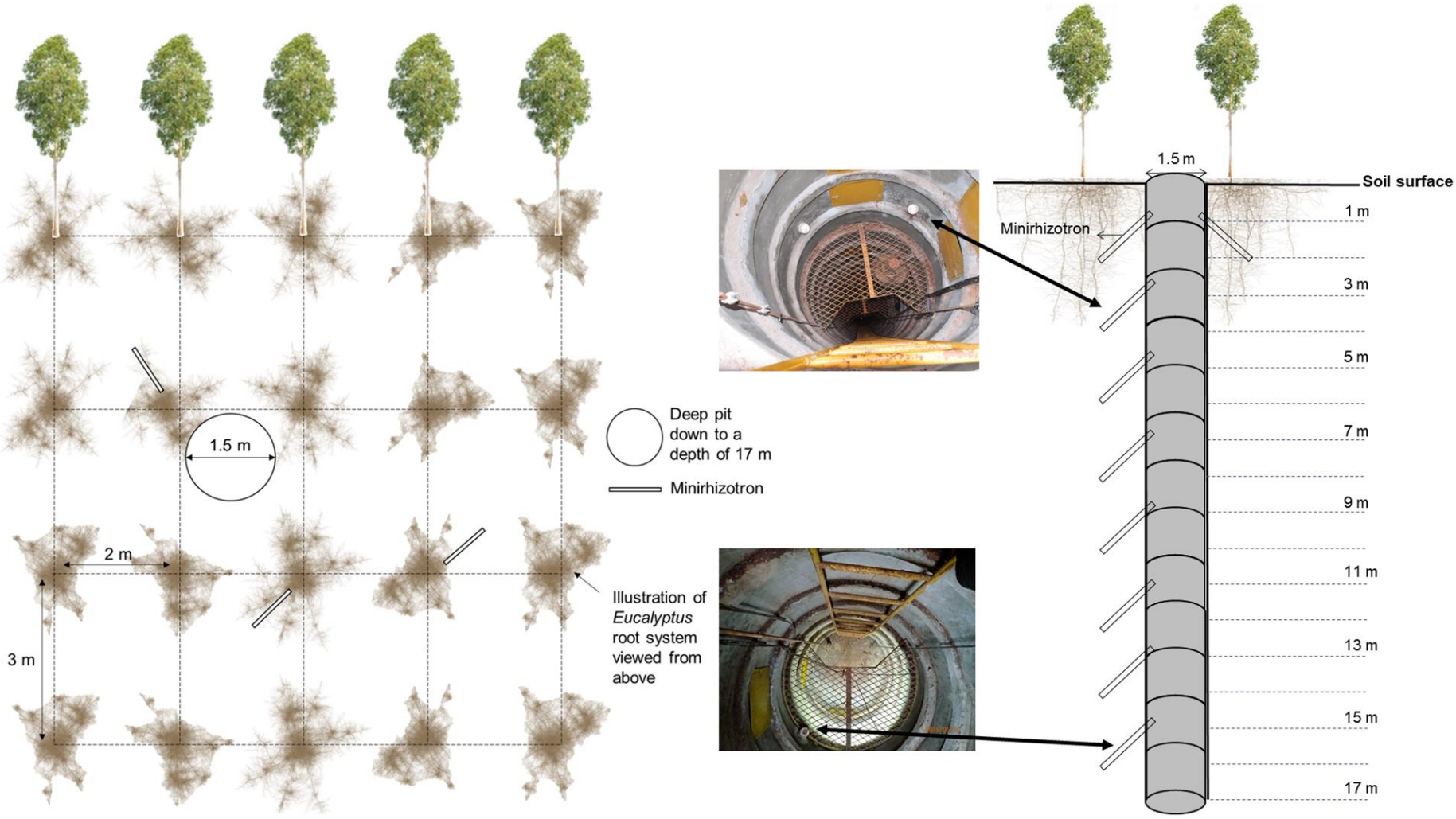

Figure V. 1: Layout of the position of the pit in the +W treatment of block 1 and distribution of minirhizotron tubes at the soil surface (n=3) and in the permanent pit from 1 m (n=2) down 17 m (n=1 per m in depth). The same set-up was used for the -W treatment.

V.2.6 Root image processing

WinRhizoTron™ software (Régent Instrument Inc., Quebec, Canada) was used to analyze more than 24,000 root images taken in the minirhizotrons. This software was chosen as it allowed the analysis of large number of images and overlayed the images to visualize the time evolution of the roots throughout the tubes. This manual root measurement program estimated the length and diameter of each root observed and stored each data item in a text file. By comparing two consecutive images chronologically, it was possible to estimate changes in root length and phenology features. Root mortality was evaluated based on the absence of growth over the entire study period up to the last images, when roots turned black and presented clear signs of decomposition (Germon et al., 2016; Lambais et al., 2017). For each fine root (diameter < 2 mm) we recorded the time of the first appearance, the diameter, the length over time, and the time of disappearance. In this study, we considered only root appearing during the study period.

V.2.7 Root length calculations

As described in Germon et al. (2016) and Lambais et al. (2017) the following metrics were used to estimate root production and the root elongation rate: living and dead root length production (LP, cm m^{-2}) was calculated for each root as the individual root length (living or dead) at time t divided by the observed soil area of each image. The cumulative living or dead root length production (CLP, cm m^{-2}) was calculated summing, at each time t, the individual length of all the roots observed, divided by the observed soil area of each image. The individual root elongation rate (RER, cm day^{-1}) was calculated as the difference in individual root length observed between two consecutive sessions (t and t_{-1}) divided by the time in days between t and t_{-1}. The daily root elongation rate (DRER, cm m^{-2} day^{-1}) was calculated by summing, at each time t, each individual root elongation rate of all the roots observed, divided by the observed soil area of each image. The mean root elongation rate (MeanRER, cm day^{-1}) and the maximum root elongation rate (MaxRER, cm day^{-1}) were calculated as the mean and the maximum of individual root elongation rates considering all the roots growing during the study period. LLP, CLP, RER, DRER, MeanRER and MaxRER were estimated for each treatment (+W, -W and NH), each soil layer and between consecutive image sessions over the entire study period.

V.2.8 Root sampling

In order to check the consistency of fine root dynamics in deep soil layers observed by the minirhizotron method, total fine root biomass, fine root length and fine root area down to the water table were measured in October 2017, i.e. 1.5 years after the clear-cut in the coppices (+W and -W) and 7.5 years after planting in NH. Fine roots (diameter < 2 mm) were sampled down to 17 m in the two treatments (+W and -W) inside the three blocks and in the non-harvested (NH) treatment inside one block. Four trees were randomly selected in each plot and soil samples were collected around each tree between the topsoil and a depth of 2 m and around 2 other trees between depths of 2 and 17 m (i.e. 12 sampling points down to a depth of 2 m and 6 further down to a depth of 17 m in the +W and -W treatments and 4 sampling points down to a depth of 2 m and 2 down to a depth of 17 m in the NH treatment). At each sampling position, soil layers at 0-0.2, 0.2-0.5, 0.5-1, 1-1.5, 1.5-2 m were collected using a cylindrical auger with an inner diameter of 4.5 cm. The Brazilian "cavadeira" tool, a cylindrical auger with an inner diameter of 9 cm and a length of 30 cm, was used to collect soil cores every 50 cm from 2 m down to a depth of 17 m. From each soil core, about 1.5 and 2 kg of soil was collected using the same methodology as described in Germon et al. (2018) and Christina et al. (2011b). To avoid contamination of the collected soil samples, only soil blocks from the inner part of the auger were considered. Total fresh soil mass was measured, and a sub-sample of 5 g was weighed in the laboratory to estimate the soil water content by drying the sample at 105°C for 72 h. Each soil sample was identified and stored at 4°C before being processed a maximum of 1 week after sampling. Living roots (i.e. living stele, bright color and elasticity) and dead roots (i.e. by sight, touch and flotation) > 1 cm long were carefully separated by hand after gentle washing away of the soil with tap water on a sieve with a mesh size of 0.5 mm. For the 0-0.2, 0.2-0.5 and 0.5-1 m soil layers, 10 % of the weight of each soil sample was used to estimate the mass of extremely fine roots (i.e. length < 1 cm). For the other soil layers 100% of the weight was considered. Living and dead roots more than 1 cm in length were scanned using a double-sided scanning procedure at a resolution of 800 dpi. Then, living roots and dead roots over > 1 cm in length and fragments of living and dead roots less than 1 cm in length were dried for 72h at 65° C and weighed (±0.1 mg). For the upper layers where only 10% of the weight of the soil was screened for living and dead roots < 1 cm in length, the mass of fragments was multiplied by 10 to estimate the mass of fragments of the whole soil sample. Root weight was then obtained for each soil layer, sampling position, treatment and block.

V.2.9 Root trait calculations

The fine root scans obtained where analyzed using WinRhizo Version Pro V. 2009c software (Régent Instruments Inc., Quebec, Canada) to estimate root lengths and areas per soil layer, sampling position, treatments (+W, -W and NH) and blocks. As described in Germon et al. (2018) the following metrics were used to estimate fine root traits. The specific root length (SRL, m g^{-1}, i.e. total length of scanned roots divided by their dry mass) and specific root area (SRA, cm^2 g^{-1}, i.e. total area of scanned roots divided by their dry mass) were calculated for each soil sample. Fine root mass density (g kg^{-1} soil) was calculated as the total root dry mass divided by the dry weight of the soil used for root separation. Fine root density (FRD, g dm^{-3} soil) was obtained by multiplying fine root mass density by the soil bulk densities (measured by the standard core method down to a depth of 17 m in each treatment). Fine root biomasses (FRB, g m^{-2}) were calculated in each soil layer multiplying the mean fine root density by the soil layer volume (dm^3). The root area index (RAI, m^2 m^{-2}) was calculated as the surface area and length of fine roots divided by the sampled soil area respectively. FRD, FRB, SRL, SRA, and RAI were obtained for each soil layer from the soil surface down to a depth of 17 m, for the sampling position, +W, -W and NH treatments, and blocks.

V.2.10 Statistical analyses

Linear mixed-effect models were used to test the effect of soil depth, treatment and the interaction between depth and treatment on cumulative length production (CLP), root elongation rate (RER), daily root elongation rate (DRER), mean root elongation rate (MeanRER), maximum root elongation rate (MaxRER), fine root density (FRD), fine root biomass (FRB), specific root length (SRL), specific root area (SRA), root area index (RAI) and root diameter for the whole soil profile. Blocks were considered as random effects and residues were modeled by a first-order autoregressive correlation model to account for the correlations between soil depths. Two-way analyses of variance (ANOVAs) were used to assess the effect of treatments and blocks for individual soil layers on CLP, RER, DRER, MeanRER, MaxRER, FRD, FRB, SRL, SRA, RAI and root diameter. Measurements within a given soil layer were considered independent, since the sampling positions were located near different trees in each treatment and plot. The homogeneity of variances was verified, and log-transformations were used when the residuals did not follow a normal distribution. Tukey's post-hoc Honest Significant Difference (HSD) was used to determine the significant differences between treatments. R software version 3.4.4 (Team R 2013) was used for all the calculations and

statistical analyses with a 5% significance level. The “lmerTest” package was used to perform the linear mixed-effect models (Kuznetsova et al., 2017).

V.3 Results

V.3.1 Tree growth

Initial vertical growth was faster in the coppices than in the replanting: nine months after harvesting (in February 2011 for the replanting, and February 2017 for the coppices), mean tree height was 1.3 m and 1.5 m in the +W and -W replanted plots and was 2.7 m and 3.1 m in the +W and -W coppice plots, respectively (Figure V. 2). There were no significant differences in vertical growth between the +W and -W treatments for either replanting or coppice in the first 3 years after harvesting.

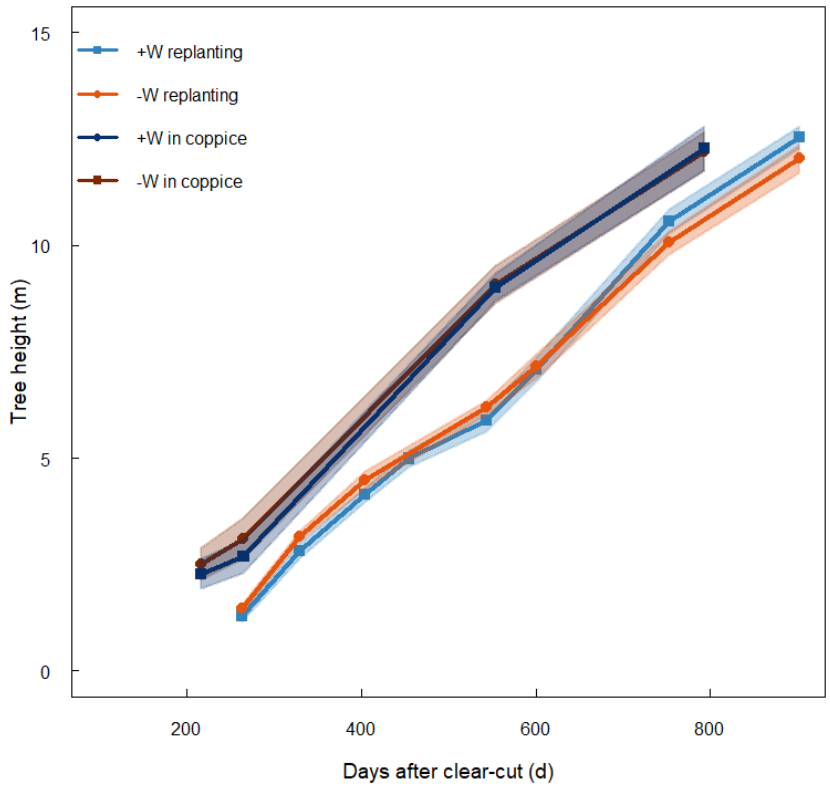

Figure V. 2: Vertical growth (m) over the first 2.5 years after harvesting of the previous stands for the replanting (June 2010-June 2016) and the coppice (from June 2016) in the undisturbed rainfall plots (+W, blue) and in the plots with 37% of throughfall excluded by plastic sheets (-W, orange). Standard errors are shown (shaded area).

V.3.2 Volumetric soil water contents (SWCs)

In both the +W and -W treatments, rainfall events led to large variations in SWC (Figure V. 3). At a depth of 0.5 m, SWC ranged from 5.9% to 15.8% in -W and from 7% to 19.4% in +W. At a depth of 14 m, SWC ranged from 9.9% to 10.9% in -W and from 11.3% to 18.1% in +W. Over the study period, throughfall reduction led to a sharp decrease in SWC, on average, by 12.9 ± 4.9% from the topsoil to a depth of 16 m. The design turned out to be efficient in mimicking two contrasting rainfall regimes. At the end of the rainy season, the mean SWC values of the soil profile in April 2016, i.e. 2 months before the clear-cut, were 12.5% and 14.0% in -W and +W, respectively and in April 2017, i.e. 10 months after the clear-cut, there were 14.4% and 16.2% in -W and +W, respectively. After clear-cutting, the soil profiles were recharged with water due to the interruption of tree transpiration. In +W, the gravitational soil solutions reached a depth of 16 m only 10 months after clear-cutting, while in -W they reached a maximum depth of only 12 m 13 months after clear-cutting. Gravitational soil solutions did not reach the water table in -W over the study period (one year before the clear-cut and the first 2 years of coppice management).

V.3.3 Fine root length production

The highest cumulative root length production (CLP) over the study period was at depths > 4 m, in both +W and -W (Figure V. 4). In –W, CLP reached about 19 m m^{-2} at a depth of 13.5-14.8 m, and only 3.9 m m^{-2} in the upper 2.3 m of the soil profile. In +W, the highest CLP was found in an intermediate soil layer (5.5-6.8 m deep) with a CLP of 18.9 m m^{-2}. CLP reached about 12 m m^{-2} in very deep soil layers (13.5-14.8 m deep) and only about 2 m m^{-2} in the upper 2.3 m of the soil profile in +W.

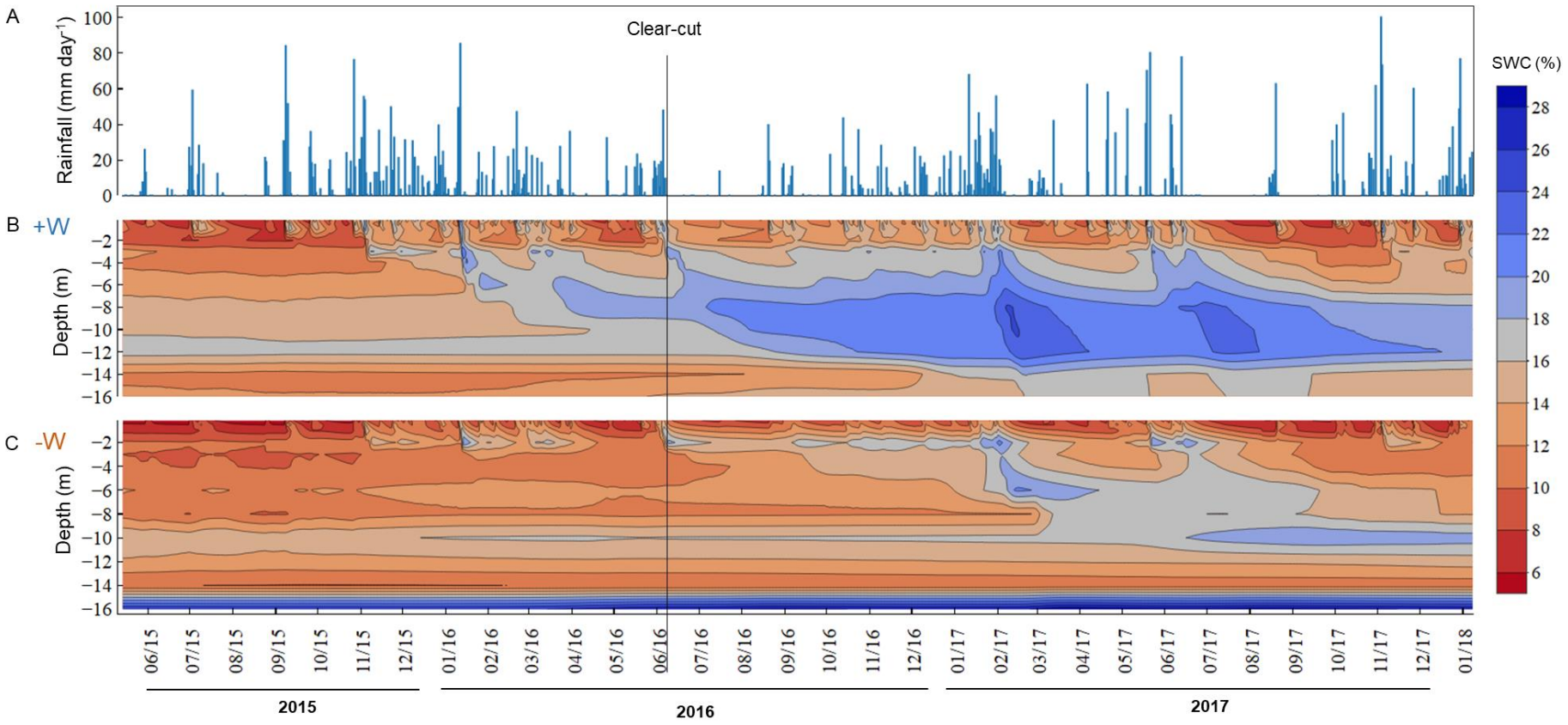

Figure V. 3: Daily rainfall (A) and soil water content (SWC, %) within the soil profiles down to a depth of 16 m in the undisturbed rainfall plots (+W, B) and in the plots with 37% of throughfall excluded by plastic sheets (-W, C) from May 2015 to January 2018. SWC graphical representation is a contour line interpolation obtained with a marching square algorithm. R software version 3.4.4 and the "plotly" package version 4.8.0 were used. The clear-cut date is indicated by a vertical line. The stand was coppice-managed after the clear-cut.

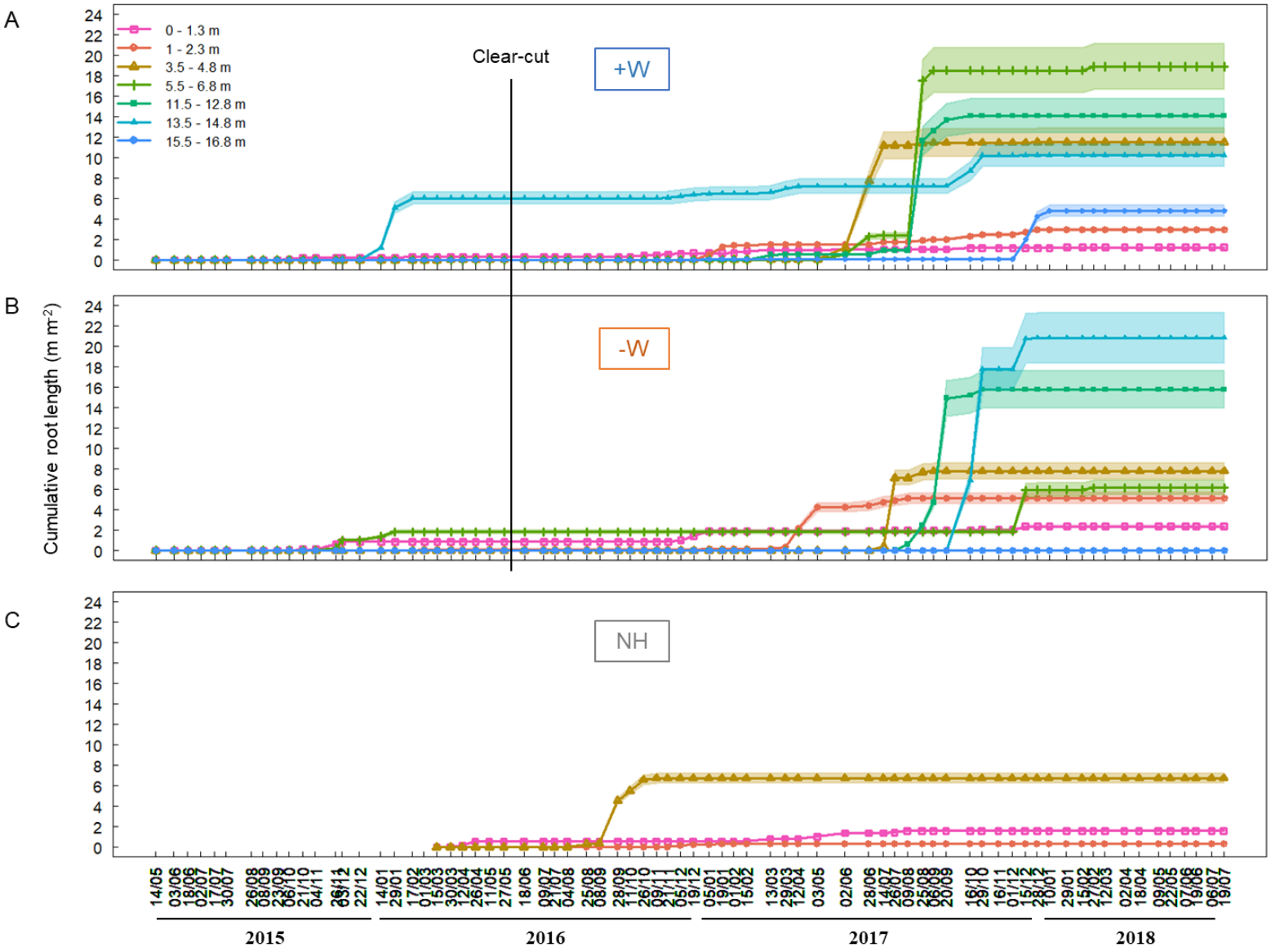
A
B
C
0 - 1.3 m
1 - 2.3 m
3.5 - 4.8 m
5.5 - 6.8 m
11.5 - 12.8 m
13.5 - 14.8 m
15.5 - 16.8 m
Clear-cut
+W
-W
NH
Cumulative root length (m m-2)
2015
2016
2017
2018

Figure V. 4: Cumulated root length production (CLP) on minirhizotron tubes (m m^{-2} of minirhizotron area) measured every 14 days from May 2015 to July 2018 in soil layers 0–1.3 m, 1–2.3 m, 3.5-4.8 m, 5.5–6.8 m, 11.5-12.8 m, 13.5–14.8 m and 15.5-16.8 m in the undisturbed rainfall plot (+W, A), in the plot with 37% of throughfall excluded by plastic sheets (-W, B) and in the non-harvested plot (NH, C). Standard errors are shown (shaded area). The clear-cut date is indicated by a vertical line. The stands were coppice-managed after clear-cutting in +W and -W. The monitoring of CLP started in May 2015 in the +W and -W plots, and in March 2016 in the NH stand, where only the first three layers (0-1.3 m, 1-2.3 m and 3.5-4.5 m) were sampled.

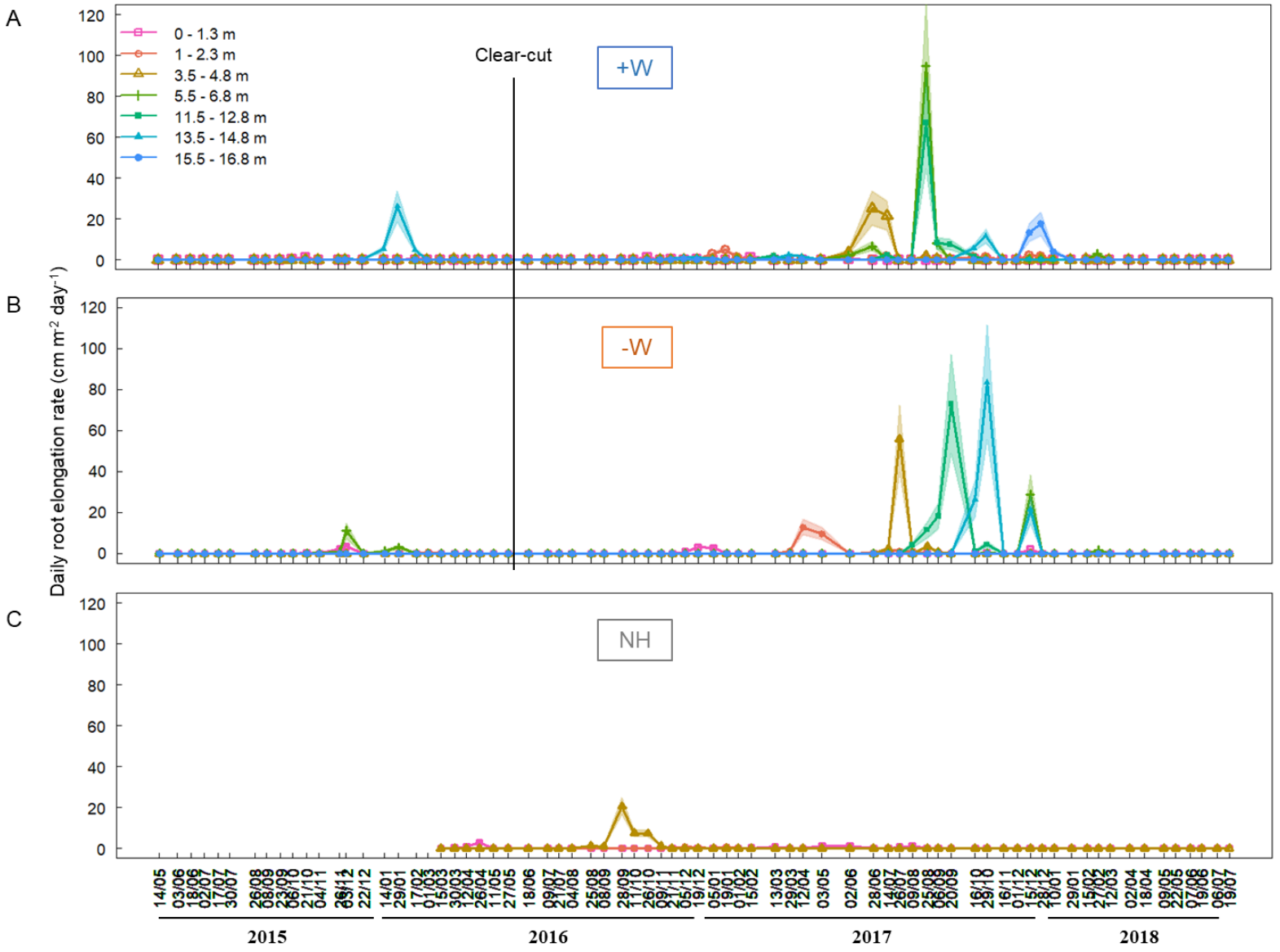
A
B
C
0 - 1.3 m
1 - 2.3 m
3.5 - 4.8 m
5.5 - 6.8 m
11.5 - 12.8 m
13.5 - 14.8 m
15.5 - 16.8 m
Clear-cut
+W
-W
NH
Daily root elongation rate (cm m-2 day-1)
2015
2016
2017
2018

Figure V. 5: Daily living root elongation rate (DRER) on minirhizotron tubes (cm m-2 of minirhizotron area day-1) estimated every 14 days from May 2015 to July 2018 in soil layers 0–1.3 m, 1–2.3 m, 3.5-4.8 m, 5.5–6.8 m, 11.5-12.8 m, 13.5–14.8 m and 15.5-16.8 m in the undisturbed rainfall plot (+W, A), in the plot with 37% of throughfall excluded by plastic sheets (-W, B) and in the non-harvested plot (NH, C). Standard errors are shown (shaded area). The clear-cut date is indicated by a vertical line. The stands were coppice-managed after clear-cutting in +W and -W. The monitoring of DRER started in May 2015 in the +W and -W plots, and in March 2016 in the NH stand, where only the first three layers (0-1.3 m, 1-2.3 m and 3.5-4.5 m) were sampled.

V.3.4 Fine root elongation rates

Daily root elongation rates (DRERs) were highly variable between the soil layer and the season, in both +W and -W (Figure V. 5). Fine root growth started in the top soil after the clear-cut and occurred more and more deeply over time in +W and -W. The intensity (measured as the maximum DRER) and the period of fine root growth differed depending on soil depth. DRER measured during flushes of root growth was much lower in the topsoil than at great depth in +W, -W and NH (no observation below 4.8 m in NH). At a depth of 3.5-4.8 m, flushes of DRER in NH came earlier than in clear-cut plots. In the coppices, the first flush of DRER occurred about 6 months after clear-cutting in the topsoil, 12 months after clear-cutting in the 3.5-4.8 m soil layer and 16 months after clear-cutting in the 13.5-14.8 m soil layer, in both +W and -W. Moreover, DRER sharply increased in the 15.5-16.8 m layer of +W 18 months after clear-cutting.

DRER peaks ranged from 1.5 cm m^{-2} day^{-1} in the topsoil to 94.7 cm m^{-2} day^{-1} at a depth of about 12 m in +W, and from 3.5 cm m^{-2} day^{-1} in the topsoil to 83.7 cm m^{-2} day^{-1} at a depth of about 14 m in -W. The maximum elongation rate of individual roots (MaxRER) reached 4.3 cm day^{-1} in -W and 3.0 cm day^{-1} in +W (Table V. 1) and was much higher at great depths than in the topsoil (data not shown). DRER values peaked at the end of the dry season in all the soil layers at depths > 6 m when SWC decreased in the upper soil layers (Figure V. 3, Figure V. 5). Thus, DRER values were not necessarily correlated with soil water contents for a given soil layer. A synchrony between the decrease in cumulated extractable water in the topsoil and DRER peaks was observed in -W (Figure V. 6) after the clear-cut. Successive DRER peaks appeared more and more deeply at the end of the dry season when the extractable water stocks in the 0-2 m layer fell below about 80 mm.

Table V. 1: Mean diameter (mm), maximum elongation rate of individual roots (cm day^{-1}), number of roots observed and root mortality over 3 years in the minirhizotron images across all the soil layers, from the surface to the water table at a depth of about 17 m, in the undisturbed rainfall plot (+W) and the plot with 37% of throughfall excluded by plastic sheets (-W).

	+W	-W
Mean diameter (mm)	0.61 ± 0.31	0.52 ± 0.28
Maximum root elongation rate (cm day^{-1})	3.0	4.3
Number of roots observed	12,247	14,118
Mortality (%)	7.4	5.7

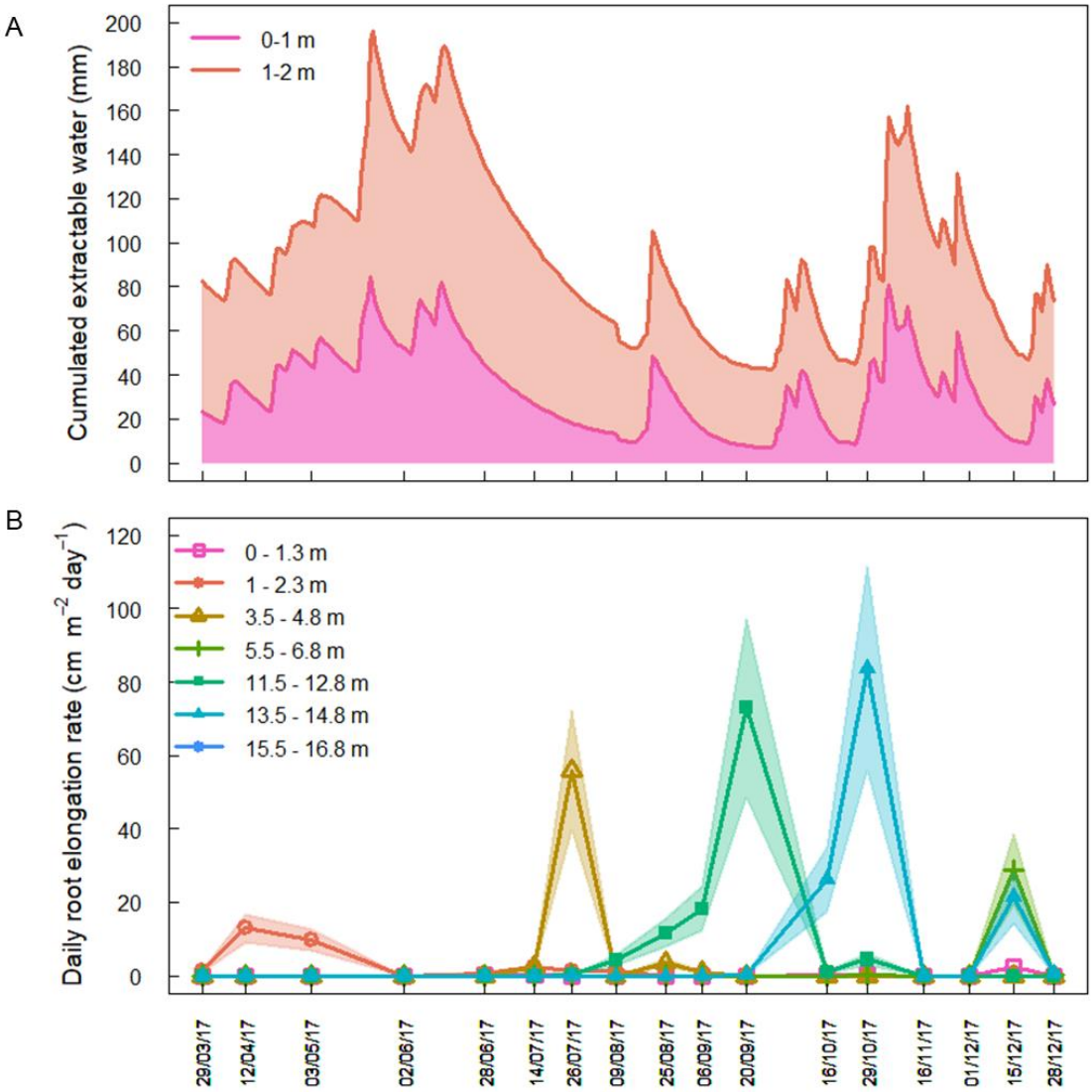

Figure V. 6: A) Time course of soil extractable water (mm) in soil layers 0-1 m and 1-2 m, in the -W (37% of throughfall excluded by plastic sheets) coppice of block 1, from March 2017 (10 months after the clear-cut) to December 2017. The mean daily values of soil extractable water were estimated from semi-hourly SWC measurements. B) Daily living root length production on the surface of minirhizotron tubes (cm m^{-2} of minirhizotron area day^{-1}) estimated over the same period and same plot as a), at two-week intervals, in soil layers 0–1.3 m, 1–2.3 m, 3.5-4.8 m, 5.5–6.8 m, 11.5-12.8 m, 13.5–14.8 m and 15.5-16.8 m. Standard errors are shown (shaded area).

V.3.5 Fine root distributions

Auger sampling carried out on the same date in the NH plot and in the +W and -W coppices 1.5 years after clear-cutting showed a similar pattern of deep rooting, whatever the treatment (Figure V. 7). Fine root densities dropped below a depth of 20 cm, then decreased gradually

down to the water table in NH, +W and -W. Fine roots where found down to 17 m in all treatments. Total fine root biomass down to a depth of 17 m was 1,078 g m^{-2} in NH, 1,017 ± 301 g m^{-2} in +W and 1,266 ± 363 g m^{-2} in -W (Table V. 2). Fine root densities were about 25% higher in -W than in +W and NH in most of the soil layers. At depths > 7 m, fine root densities were at least twice as high in -W as in +W and NH.

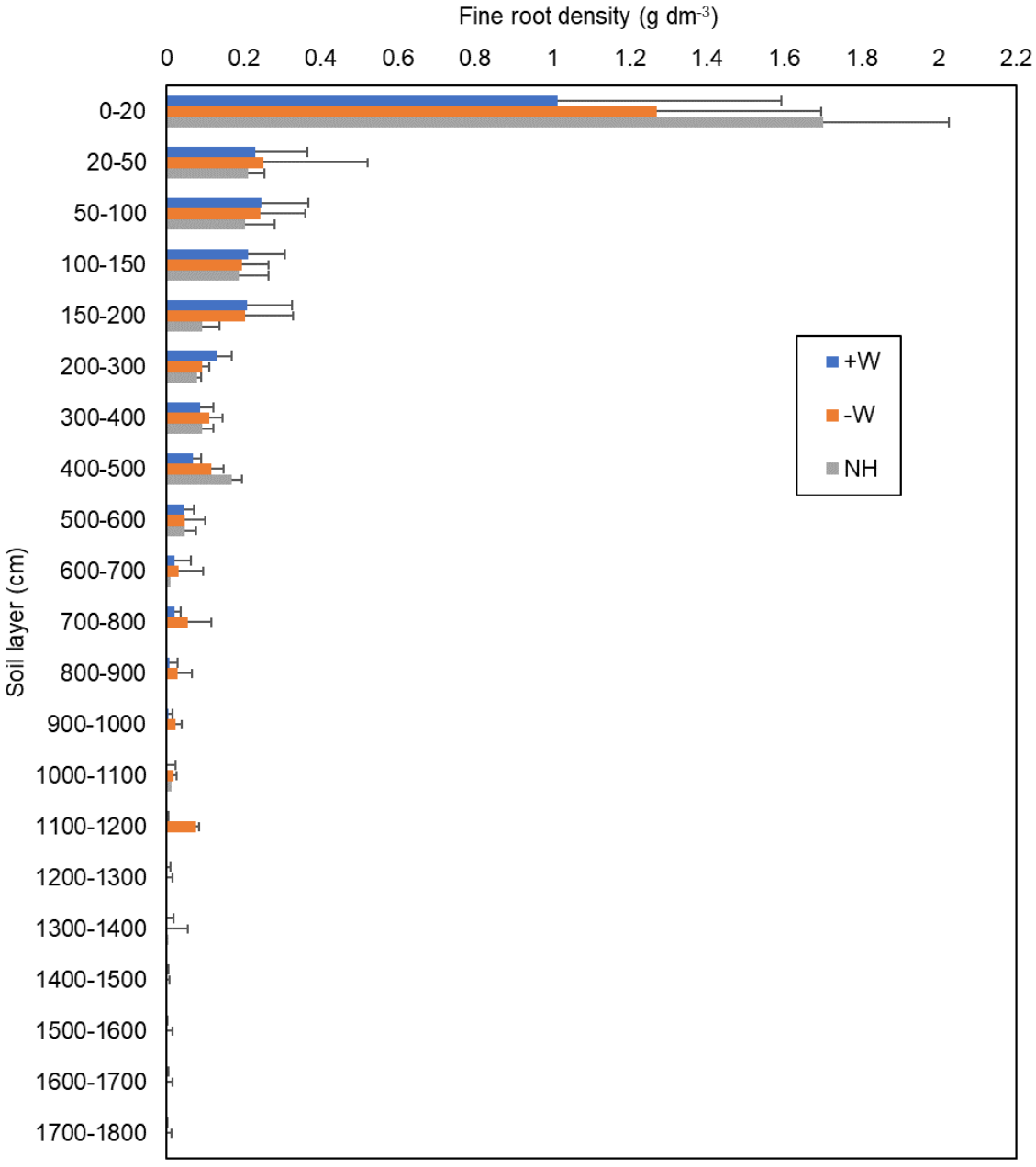

Figure V. 7: Mean fine root densities down to the root front in the undisturbed rainfall plots (+W, blue), in the plots with 37% of throughfall excluded by plastic sheets (-W, orange) and in the non-harvested plot (NH, gray). Standard deviations between blocks (n = 3) for -W and +W and between pseudo replicates in a single block for NH are shown.

Table V. 2: Total living fine root biomass (g m^{-2}), total fine root necromass (g m^{-2}), mean specific root length (m g^{-1}), mean specific root area (cm^2 g^{-1}) and mean root diameter down to a depth of 17 m in the undisturbed rainfall plots +W, in the plot with 37% of throughfall excluded by plastic sheets (-W), both in coppices and 1.5 years after the clear-cut, and in the non-harvested plot (NH), i.e. a 7.5-year-old tree plantation. Standard deviations are indicated. Different lower-case letters indicate significant differences between treatments +W and -W ($p < 0.05$). NH was not included in statistical analyses because the three soil coring replicates were located in the same plot.

	+W	**-W**	**NH**
Fine root biomass (g m^{-2})	1,016.5 ± 362.8	1,265.8 ± 301.4	1,078.3 ± 83.9
Fine root necromass (g m^{-2})	163.2 ± 55.9	167.9 ± 31.3	139.1 ± 17.7
Specific root length (m g^{-1})	30.6 ± 6.9 b	34.4 ± 14.1 a	28.8 ± 7.6
Specific root area (cm^2 g^{-1})	267.5 ± 55.7 b	305.3 ± 150.1 a	258.9 ± 92.6
Root diameter (mm)	0.32 ± 0.08 b	0.36 ± 0.13 a	0.31± 0.08

V.3.6 Fine root traits

The total root area index (RAI) was significantly higher in –W (32.8 m^2 m^{-2}) than in +W (26.5 m^2 m^{-2}) (Figure V. 8). Similar RAI values in the NH stand (26.8 m^2 m^{-2}) and in the +W coppice suggested that the effect of clear-cutting on fine root dynamics was low, in agreement with elongation and mortality rates observed in the upper 4 m on minirhizotron tubes. RAI in the 0-1 m surface soil layer accounted for only one third of the total RAI down to the water table in NH, +W and -W (about 11 m^2 m^{-2} on average). Significant differences between +W and -W were found at depths > 5 m (Figure V. 8). In the 5-10 m soil layers, RAI was 5.0 m^2 m^{-2} in -W and 2.8 m^2 m^{-2} in +W. At depths > 10 m, RAI was 3.2 m^2 m^{-2} in -W and only 0.4 m^2 m^{-2} in +W.

In the NH, +W and -W treatments, depth had little effect on specific root length (SRL), specific root area (SRA) and fine root diameter (data not shown). SRL and SRA were significantly higher in -W than in +W, and similar values were observed in +W and NH, although the sampling in NH (pseudo-replication on one block) did not allow a confirmation with statistics (Table V. 2). Down to a depth of 17 m, mean SRL values were 28.8, 30.6 and 34.4 m g^{-1}, mean SRA values were 258.8, 267.5 and 305.3 cm^2 g^{-1}, and mean fine root diameters were 0.31, 0.32 and 0.36 mm in NH, +W and -W, respectively (Table V. 2).

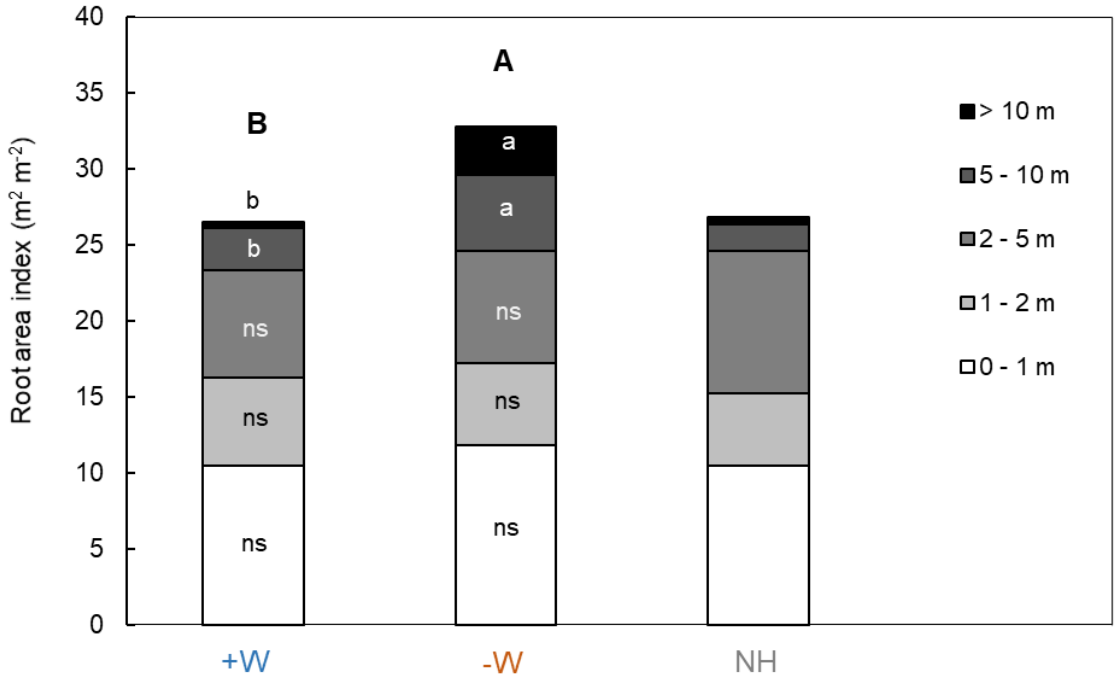

Figure V. 8: Root area index (m^2 m^{-2}) in the 0-1 m, 1-2 m, 2-5 m, 5-10 m and 10-17 m deep soil layers in the undisturbed rainfall plots (+W), in the plots with 37% of throughfall excluded by plastic sheets (-W) and in the non-harvested plot (NH). Different upper-case letters indicate significant differences between treatments for the cumulative indices and different lower-case letters indicate significant differences between treatments within each individual soil layer ($p < 0.05$). NH was not included in the ANOVA because the three soil coring replicates were located in the same plot.

V.3.7 Fine root mortality

Cumulative dead root length in the minirhizotron images over the study period of 3 years across all soil layers accounted for only 6-7% of cumulative root length production, in both +W and -W (Table V. 1). Surprisingly, dead fine root length did not increase after clear-cutting and did not differ between depths and between the +W and -W treatments. Dead fine root mass estimated 1.5 years after clear-cutting amounted to 163.2 g m^{-2} in +W and 167.9 g m^{-2} in -W (Table V. 2).

V.4 Discussion

The main novelty of our results lies in the first direct observation of fine root phenology at depths of more than 10 m and derived knowledge raised from a comparison between the treatments. The consequences of tree harvesting on fine root dynamics in coppice-managed plantations raise specific questions never studied before along a deep tropical soil profile. Since the 1980s, the minirhizotron technique has been widely used to study fine root dynamics and turnover (Graefe et al., 2008; Maeght et al., 2013). Many studies have shown that the soil environment close to minirhizotrons can be modified relative to undisturbed soil (Majdi & Nylund, 1996), which is likely to influence fine root dynamics. However, in our study the very low root mortality rates after clear-cutting revealed by minirhizotron monitoring was consistent with the small amounts of dead fine roots measured by soil coring. Despite some unavoidable limitations, direct observations from minirhizotron tubes or field rhizotrons is for now the most accurate way of studying fine root phenology in situ (Dipesh & Schuler, 2013; Radville et al., 2016a).

V.4.1 Root behavior in response to drought

The main purpose of our study was to investigate whether fine roots explore very deep soil layers and do so more intensively when trees are subjected to prolonged drought periods. This study, carried out in a throughfall exclusion experiment and including a non-harvested plot, made it possible to assess the effect of clear-cutting under two contrasting rainfall regimes. In agreement with our first hypothesis, *Eucalyptus* trees responded to drought by increasing fine root densities at great depth. Previous studies in Brazil showed that *Eucalyptus* trees have the ability to explore very deep layers in soils without hindrance to root growth (Christina et al., 2011b; Pinheiro et al., 2016b). In a survey of 62 tropical tree species, Markesteijn and Poorter (2009) showed that trees increase belowground biomass and the proportion of deep roots in response to dry conditions. Root growth in deep soil layers can increase the amount of water available to sustain tree growth, which could be a key advantage for eucalypt trees in coping with severe drought events (Christina et al., 2017b).

After clear-cutting, fine root growth at more than 4 m deep was spectacular at the end of the dry season, whatever the water supply regime. Lambais et al. (2017) also showed flushes of fine root growth down to a depth of 6 m at the end of dry periods in a Brazilian eucalypt plantation. Fine root growth in deep soil layers was initiated when the extractable water content

in the 0-2 m soil layer fell below a threshold of about 80 mm, which suggests that soil water content in a particular soil layer was not the main driver of fine root growth. Endogenous and exogenous factors are major drivers of fine root phenology (Abramoff & Finzi, 2015; Moroni et al., 2003; Tierney et al., 2003), but are difficult to disentangle in deep soil layers. The successive flushes of fine root growth from the topsoil to the deepest soil layers at the end of the dry season and the first months after the onset of the rainy season in +W and -W might have resulted from a rapid exhaustion of water resources in the topsoil, inducing tree roots to grow deeper in the soil (Billings, 2015; Schenk & Jackson, 2002). Furthermore, a strong increase in sugar sap concentration and sugar allocation belowground has been shown in forests during dry periods (Pate & Arthur, 1998; Scartazza et al., 2015), which could be a physiological response to a rapid exhaustion of water in the top soil and could help to explain the root growth in deep soil layers at the end of dry periods in our study.

In agreement with our second hypothesis, *Eucalyptus* trees facing drought adapted their root morphology to maximize the root surface area to take up limited resources. SRL and SRA were higher in –W than in +W, which in combination with higher fine root densities led to a much higher root area index. In a meta-analysis, Ostonen et al. (2007b) showed that SRL response to drought varies greatly between species. While Arend et al. (2011) did not observe any significant change in SRL, root area index and root tissue density for oak trees (*Quercus sp.*) exposed to drought, Olmo et al. (2014) showed an increase in SRL and root tissue density under drought conditions for 10 tree species. *Eucalyptus grandis* trees coped with drought in our experiment by increasing their capacity to take up soil resources for a relatively low investment in belowground biomass. A large increase in SRL and SRA was recently shown throughout deep soil profiles down to 17 m deep for *Acacia mangium* Wild and *E. grandis* roots in response to the mixture relative to monospecific stands (Germon et al., 2018). Our study showed that *Eucalyptus* trees can modify fine root traits to enhance resource capture and the exploration of very deep soil layers to survive in a context of climate change, which contributes to maintaining water uptake during dry periods (Brunner et al., 2015; Christina et al., 2017b).

V.4.2 Key role of deep roots in coppice management

Contrary to our fourth hypothesis, relatively few roots were lost by mortality (< 10% out of all the roots observed over 3 years), which suggested that most of the root system remained functional after clear-cutting. The effect of clear-cutting on fine root mortality remains unclear in coppice-managed forest plantations. Unlike our observations, sequential coring in the 0-1 m

soil layer showed a decrease in fine root density after harvesting coppice-managed *E. grandis* stands in Brazil (Mello et al., 2007). Wildy and Pate (2002) also showed high fine root mortality rates after cutting the trees in plantations of *Eucalyptus kochii* Maiden & Blakely subsp. plenissima Gardner (Brooker), but the root system superstructure was sustained down to a depth of at least 4 m. Teixeira et al. (2002) showed from sequential coring in the 0-0.6 m soil layer that fine-, medium- and coarse-root biomasses increased over time after harvesting coppice-managed *Eucalyptus urophylla* S. T. Blake trees. Using the minirhizotron technique, Dickmann et al. (1996) showed an absence of root mortality for coppice-managed poplar clones. Our study suggested that 6-year-old *Eucalyptus grandis* trees have enough reserves within the root system and the appropriate edaphic surrounding conditions to maintain the fine root biomass established down to the water table after clear-cutting, which probably contributed to promoting early shoot growth (Brunner et al., 2015; Drake et al., 2013).

Fine root growth after clear-cutting started in the topsoil, then continued successively in deeper and deeper soil layers, which confirmed our third hypothesis, even though the lapse of several months after clear-cutting was not expected. This pattern might have resulted from high nutrient and water availabilities in the upper layers after harvesting *Eucalyptus* stands due to an interruption of tree water uptake and fertilizer application (Laclau et al., 2010). The asynchrony within the root system, delaying root growth in deep soil layers, might be a strategy for maximizing the water and nutrient uptake needed to meet the high demand of these fast-growing trees. Root growth occurred at a depth of 14 m less than one year after clear-cutting of coppice-managed *E. grandis* trees, while the maximum depth reached by *E. grandis* roots one year after planting is about 7 m in very deep soils (Christina et al., 2011b; Laclau et al., 2013a). Deep roots can provide access to large amounts of water stored in the subsoil after clear-cutting and small fine root densities in deep soil layers can be sufficient to take up substantial amounts of water (Christina et al., 2017b). Low fine root mortality rates after clear-cutting suggest that coppice-management of *Eucalyptus* plantations might be a promising option for coping with water scarcity, since the pre-existing root system can provide access to water stored throughout deep soil profiles.

V.4.3 Carbon sequestration and implication for the management of eucalypt plantations

Surprisingly, cutting the trees did not lead to an increase in root mortality throughout the soil profile, whatever the water supply regime. Fine roots play an active role in carbon (C) cycling in forest ecosystems, through respiratory processes, exudation and root mortality (Balesdent &

Balabane, 1996; Marsden et al., 2008; Strand et al., 2008). The consequences of deep rooting on subsoil C stocks in tropical planted forests and other forest ecosystems remain poorly known (Gao et al., 2018; Harper & Tibbett, 2013; Meyer et al., 2018; Nepstad et al., 1994). On the one hand, the supply of fresh carbon might promote the activity of microbes and affect the stability of pre-existing organic matter through a "priming effect" (Derrien et al., 2014a; Fontaine et al., 2007; Kuzyakov et al., 2000). Promoting the mineralization of ancient C would result in high emissions of carbon dioxide (CO_2) under aerobic conditions and of methane (CH_4) under anaerobic conditions. However, a complementary study in our plots showed that CO_2 and CH_4 emissions did not increase significantly after clear-cutting in the coppice-managed stands compared to the non-harvested stand (Germon et al. in prep.). Gas emission rates after clear-cutting in our experiment were therefore consistent with the low rates of root mortality observed. On the other hand, deep roots might also contribute to sequestrating large amounts of C in soil. Total below-ground carbon allocations account for about 20-30% of gross primary production in *Eucalyptus* plantations (Epron et al., 2012; Nouvellon et al., 2012a; Ryan et al., 2010). Fine root elongation rates were higher at great depth compared to the topsoil, with an increase in fine root density after the clear-cut in very deep horizons. Moreover, microbial biomass is lower in deep soil layers than in the topsoil, which in combination with oxygen limitations could enhance C sequestration as a result of low mineralization rates (Rumpel & Kögel-Knabner, 2011; Taylor et al., 2002). C from roots is generally retained more in the soil than C from aboveground litter, which is more affected by physicochemical processes and also due to structural composition differences between leaves and roots (Menichetti et al., 2015; Rasse et al., 2005; Schmidt et al., 2011). Further studies are needed to assess whether the management of *Eucalyptus* plantations in very deep soils could be an effective option to help mitigate the increase in CO_2 in the atmosphere.

V.5 Conclusions

The fine root phenology of coppice-managed *Eucalyptus* trees under contrasting water supply regimes revealed unexpected low rates of root mortality. The early growth of the sprouts after cutting the trees benefited from the root system established over the previous rotation cycle, and the asynchrony of fine root growth depending on depth highlighted tree plasticity in response to soil conditions. Establishing deep root systems in tropical planted forests could help trees withstand the long drought periods expected under climate change in many tropical regions. Our study suggested that coppice management might be an interesting option in

tropical *Eucalyptus* plantations, both to improve tree tolerance to drought and store carbon at great depth in the soil.

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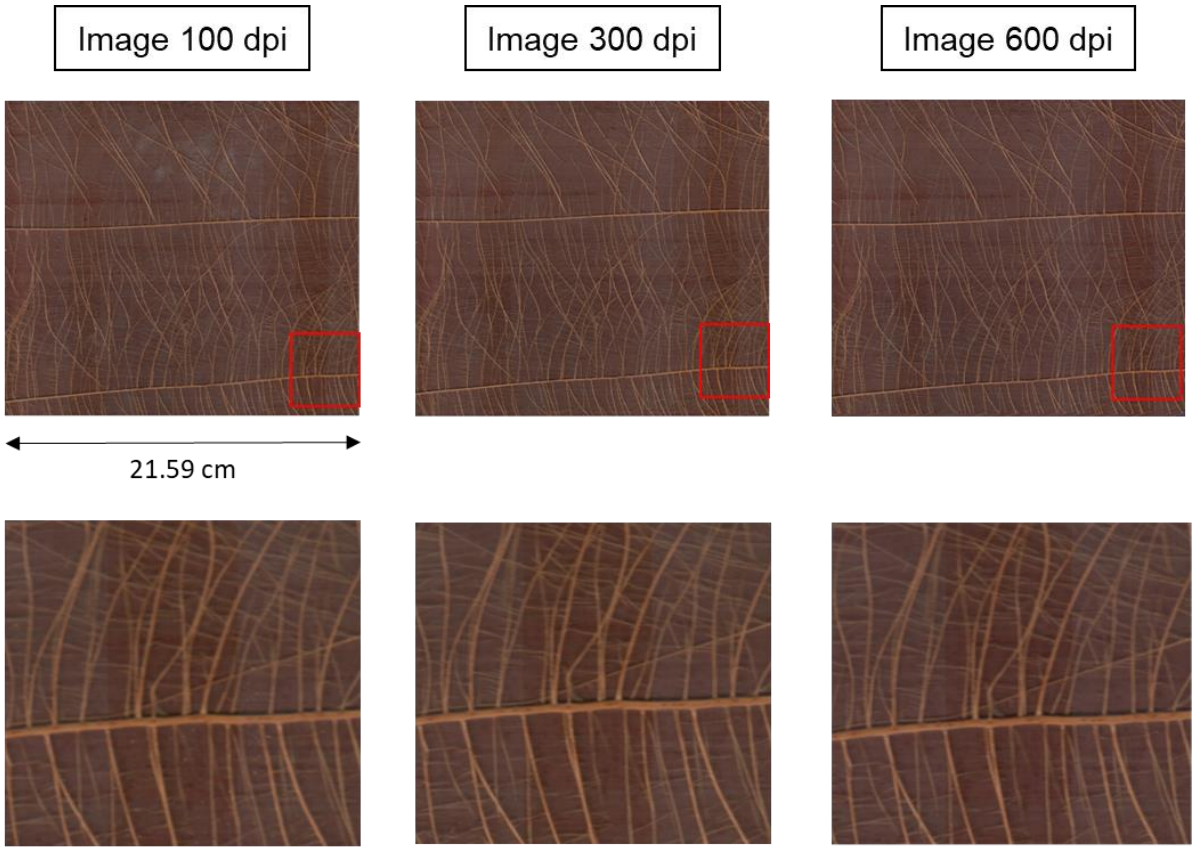
Appendix

Appendix V. 1: Images scanned in the minirhizotron installed at 11.5 m depth in the deep permanent pit of the undisturbed rainfall plot between September and November 2017. A spectacular fine root growth was observed in deep soil layer.

Appendix V. 2: Images scanned in minirhizotron tube comparing different image quality (100 dpi, 300 dpi and 600 dpi).



Appendix V. 3: Images scanned in the minirhizotron installed at 10 m depth in the deep permanent pit of the undisturbed rainfall plot in May 2015 (left) and July 2018 (right). No root growth and mortality were observed in 3 years in this tube.

Appendix V. 4: Images scanned in the minirhizotron installed at 14 m depth in the deep permanent pit of the undisturbed rainfall plot in March 2016 (left), March 2017 (center) and March 2018 (right). No root mortality was observed even after the clear-cut and a root grow 10 months after the clear-cut was observed at 14 m depth in this tube.

What to remember from Chapter 5?

The main results of this chapter are summarized in the following Table V. 3.

Table V. 3: Main results obtained in the experimental set up on root traits categories in deep soil layers, subjected to drought and in response of clear-cutting and coppice management. ↗ indicates a positive effect, ↘ indicates a negative trend and → indicates predominantly no effect.

Root traits categories	**Root trait**	**Depth**	**Drought**	**Clear-cut**
Growth	Fine root density (g dm^{-3})	↘	↗	→
	Fine root biomass (g m^{-2})	↘	↗	→
	Fine root necromass (g m^{-2})	↘	→	→
	Root elongation rates (cm day^{-1})	↗	↗	↗
Architectural	Root area index (m^2 m^{-2})	↘	↗	→
	Specific root length (m g^{-1})	→	↗	→
	Specific root area (cm^2 g^{-1})	→	↗	→
	Fine root diameter (mm)	→	↗	→

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CHAPTER 6: CONSEQUENCES OF CLEAR-CUTTING AND DROUGHT ON CO_2, CH_4 AND N_2O PRODUCTIONS THROUGHOUT DEEP SOIL PROFILES IN COPPICE-MANAGED EUCALYPT PLANTATIONS

We saw in chapter 5 that harvesting did not lead to an increase in root mortality in the stands coppice-managed, whatever the water supply regime. Fine root biomass and root area index increased about 25 and 24 % respectively in the throughfall exclusion plot compared to the control plot down to a depth of 17 m. The changes in root distribution occurred mainly at depth > 7m. Therefore, the huge soil volume explored by fine roots might lead to significant CO_2, N_2O and CH_4 productions within the entire rooting soil profile. A deeper root system could increase greenhouse gases emissions through an increase in production, respiration, exudation and decomposition processes.

The central objective of this following chapter is to assess the effect of clear-cutting and drought on greenhouse gases production throughout deep soil profiles down to the water table in coppice-managed Brazilian eucalypt plantations. These effects were monitored before and after trees harvest in a coppice-managed *Eucalyptus grandis* plantation under two contrasting water supply regimes.

This chapter presents the results of 1.8 years of greenhouse gases monitoring and will be submitted in the end of May as a scientific article to the journal Global Biochemical Cycles after revision of all co-authors. Its titled is "Consequences of clear-cutting and drought on CO_2, CH_4 and N_2O productions throughout deep soil profiles in coppice-managed eucalypt plantations". Co-authors associated with this project are Lydie Chapuis-Lardy, Yann Nouvellon, Christophe Jourdan, Agnès Robin, Joannès Guillemot, Ciro Antonio Rosolem, José Leonardo de Moraes Gonçalves, Iraê Amaral Guerrini and Jean-Paul Laclau.

Consequences of clear-cutting and drought on CO_2, CH_4 and N_2O productions throughout deep soil profiles in coppice-managed eucalypt plantations

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Key words: *Eucalyptus grandis*, greenhouse gas, deep Ferralsol profile, throughfall exclusion, coppice, Brazil

Abstract

The major factors driving greenhouse gas exchanges in forest soils (substrate supply, temperature, water content) can vary considerably with depth. Our study aimed to assess the effects of clear-cutting and drought on the temporal variability of CO_2, CH_4 and N_2O fluxes throughout very deep soil profiles in Brazilian coppice-managed eucalypt plantations. CO_2, CH_4 and N_2O surface effluxes at soil surface were measured over three years using the closed-chamber method in a throughfall exclusion experiment comparing stands with 37% of throughfall exclusion (-W) and stands without rain exclusion (+W). CO_2, CH_4 and N_2O concentrations in the soil were measured from two permanent pits down to a depth of 15.5 m in -W and +W, as well as down to a depth of 4 m in a non-harvested control plot (NH). Measurements were carried out every 2 weeks over 3 months before clear-cutting, then over 19 months in coppice. CO_2, CH_4 and N_2O effluxes were not significantly different between -W and +W and did not change after clear-cutting in the coppice-managed stands compared to non-harvested stand. CO_2 and CH_4 concentrations greatly increased with depth and N_2O concentrations remained roughly constant from the soil surface down to a depth of 15.5 m. Mean CO_2 and N_2O concentrations in -W were 20.7% and 7.6% lower than in +W, respectively, and CH_4 concentrations in -W where 44.4% higher than in +W throughout the soil profiles. A diffusivity model showed that CO_2, N_2O and CH_4 production and consumption occurred at great depths and were similar in +W, -W and NH plots. Clear-cutting did not increase CO_2, CH_4 and N_2O effluxes and productions, whatever the water supply regime. Our study suggests that the consequences of changes in rainfall regimes and silvicultural practices on greenhouse gas emissions could be minor in tropical eucalypt plantations.

VI.1 Introduction

Climate changes are expected to increase the contrast between dry and wet seasons in tropical and subtropical regions (Dai, 2011; He & Soden, 2017; Solomon et al., 2009). Climate models predict longer and more severe seasonal droughts in the future, as well as more frequent extreme rainfall events during rainy seasons (Chen et al., 2017). Changes in precipitation regimes may also have important feedback effects on climate through a modification of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) fluxes in forest soils (Davidson et al., 2004; Hashimoto et al., 2007). Understanding how precipitation changes, and especially extended drought periods, influence greenhouse gas effluxes in forest soils is crucial to improve the prediction of terrestrial biosphere models.

The balance between production and consumption of CO_2, CH_4 and N_2O in forest soils can be greatly affected by precipitation changes (Davidson et al., 2000b). Throughfall exclusion in tropical and subtropical forests has either decreased (Cleveland et al., 2010) or increased (Van Straaten et al., 2011; Wood & Silver, 2012) soil CO_2 emissions, either increased consumption (Wood & Silver, 2012) and production (Cattânio et al., 2002) of CH_4 and either lowered (Davidson et al., 2004) and increased (Cattânio et al., 2002) soil N_2O emissions. A decrease in soil water contents inhibits gas diffusion within soil profiles to the atmosphere as well as microbial production and consumption of greenhouse gases (Qi et al., 2018; Sotta et al., 2007). Root:shoot ratios generally increase in response to dry conditions as well as the proportion of deep roots (Germon et al., 2019; Markesteijn & Poorter, 2009), which can increase CO_2 production due to higher root respiration.

Production of greenhouse gases by soil microorganisms and roots can change rapidly temporally and spatially, which makes modelling efforts challenging (Courtois et al., 2018; Drewitt et al., 2005). While most studies dealing with soil greenhouse gases effluxes address the spatial and temporal variations, the vertical distribution of soil respiration is little documented in very deep soils (Drewitt et al., 2005). Trees with roots at depths of more than 10 m are common in tropical forests (Broedel et al., 2017; Nepstad et al., 1994; Saleska et al., 2007). In a Brazilian Amazon forest, the consumption of CH_4 occurred mainly in the upper 10 cm, the production of N_2O mainly in the 0-25 cm soil layer whereas CO_2 was produced down to at least a depth of 11 m (Davidson et al., 2004) . In another Amazonian forest, CO_2 production in the 0.5-3 m soil layer accounted for 17 % of the total soil CO_2 production (Sotta et al., 2007).

Even if microbial activity is low in deep soil layers (Fontaine et al., 2007), the huge soil volume explored by fine roots might lead to non-neglectable CO_2, N_2O and CH_4 productions.

Fast-growing *Eucalyptus* plantations cover more than 20 million hectares and are expanding rapidly to produced raw material for the industry (paper, wood panels, and biofuel) as well as firewood and charcoal in developing countries (Booth, 2013). In Brazil, *Eucalyptus* plantations cover about 5.6 million hectares and are mainly established on deep soils with low fertility and many plantation areas are subjected to prolonged drought periods (Keenan et al., 2015). The management of *Eucalyptus* plantations through coppice has been practiced by forestry companies due to lower production costs, shorter rotations and faster financial returns compared to replanted stands (Gonçalves et al., 2013). The root system established during previous rotations makes possible a rapid regrowth from the stump after harvesting. *Eucalyptus* fine roots can explore rapidly after planting both the superficial soil layers and very deep soil horizons (Germon et al., 2019; Laclau et al., 2013) and allocate about 30% of the gross primary productivity belowground, for root growth, root respiration and C exudation (Epron et al., 2012; Nouvellon et al., 2012; Ryan et al., 2010). Coppice management constitutes an advantage over sites with pronounced stress conditions, as the established root system preserves carbon for the next generation. However, removing the aboveground parts of trees has a great impact on carbon balance and greenhouses gases emissions of the ecosystem. Clear-cutting increases the solar radiation transmitted to the soil, raising the soil temperature and soil water contents through an interruption of stand transpiration (Londo et al., 1999; Mello et al., 2007). This may induce an increase in microbial activity, the decomposition of harvest residues left at the soil surface and enhance fine root mortality and decomposition (Mello et al., 2007; Parfitt et al., 2001).As far as we know, concentrations of greenhouse gas in deep soil layers have never been quantified in tropical planted forests in response to throughfall exclusion and coppice management.

Our study aimed to assess the consequences of contrasting rainfall regimes on CO_2, CH_4 and N_2O fluxes throughout very deep tropical soils in coppice-managed *Eucalyptus grandis* (Hill ex. Maid) stands after clear-cutting. We put forward three hypotheses: (H1) throughfall exclusion lower soil water contents, which increases gas diffusivity and modify greenhouse gases production and consumption, (H2) throughfall exclusion increases the partition of C to deep roots, which increases the production of CO_2 in deep soil layers, and (H3) fine root mortality and slash decomposition after harvesting *Eucalyptus grandis* trees managed in

coppice increase substrate availability for microbes throughout the soil profile, which increases CO_2, CH_4 and N_2O effluxes at soil surface.

VI.2 Materials and Methods

VI.2.1 Study site

Our study was carried out at the research station owned by the Luiz de Quieroz College of Agriculture (ESALQ) near Itatinga (São Paulo state, Brazil, 23°02′S 48°38′W), at an altitude of 850 m. The relief is typical of the São Paulo Western Plateau, with a topography varying from flat to hilly (slopes < 3%). The soils are very deep Ferralsols according to the ISS Working Group WRB (2015) developed on Cretaceous sandstone. Clay contents range from 160 mg g^{-1} in the topsoil to about 250 mg g^{-1} at a depth of 15 m (Christina et al., 2015). The local climate is humid subtropical (Cwa according to the Köppen classification) with a rainy season from October to May and a dry and slightly cold season from June to September. Over the last 15 years, the mean air temperature was 20°C, the relative humidity was 77% and the mean annual precipitation was 1390 mm.

VI.2.2 Experimental Layout

A split-splot experimental design was installed on 2 hectares in June 2010, with the planting of a *Eucalyptus grandis* clone used in commercial plantations (Suzano Company). Three fertilization regimes and two rainfall regimes were compared in 3 blocks to gain insight into the interaction between tree nutrition and water availability on tree functioning (Battie-Laclau et al., 2014a; Christina et al., 2015; Christina et al., 2018; Ployet et al., 2019). The experimental layout was described in detail in Battie-Laclau et al. (2014b). Each plot was made of 144 trees (12 rows with 12 trees per row, spacing 3 x 2 m) and each experimental unit consisted of 36 trees (excluding 3 buffer rows to avoid bordure effect). We studied two treatments out of the 6 treatments in the experimental design as well as a non-harvested control plot in the same stand:

- -W: plots were equipped with plastic sheets in September 2010 to exclude 37% of throughfall and fertilizers were applied to avoid any nutritional limitation of tree growth.
- +W: undisturbed rainfall and same fertilization as in -W plots
- NH: undisturbed rainfall and same fertilization as in -W and +W plots. Contrary to -W and +W, the trees were not harvested in June 2016.

Trees were planted in June 2010 and received at planting 3.3 g P m^{-2} and 200 g m^{-2} of dolomitic lime and trace elements. At 3 months of age, 0.45 mol K m^{-2} were applied as KCl and 12 g N m^{-2}. These fertilizers inputs are not limiting for the tree growth (Laclau et al., 2009). Herbicides were applied to avoid the presence of understory species. The *Eucalyptus* stands were harvested six years after planting, in June 2016, and coppice-managed thereafter. Several new shoots were regenerated from the stumps after the clear-cut and 1 or 2 shoots per stump were selected to maintain the same stocking density and prevent the growth of additional new shoots.

VI.2.3 Soil water monitoring and soil bulk density

Between February and March 2014, two deep permanent pits were excavated in the +W and -W treatments to set up sensors and to measure soil properties from the top soil down to the water table (Germon et al., 2019). The volumetric soil water content (SWC, m^3 water m^{-3} soil) was monitored through the study period at half-hourly intervals using CS616 probes (Campbell Scientific Inc., Logan, UT, USA). Probes were installed in the +W and -W treatments at a depth of 0.15, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14 and 16 m, with 3 probes at each depth in block 1. Soil bulk densities were measured by the standard core method down to a depth of 17 m in the +W and -W treatments. The total porosity was calculated from bulk density:

$$Total\ porosity = 1 - \frac{BD}{PD}$$

where BD is measured bulk density and PD is an assumed particle density of 2.65 g cm^{-3} (Davidson et al., 2006; Oerter et al., 2018; Sotta et al., 2007). The soil water content was expressed as the percentage of water filled pore space (WFPS, %) and was calculated for each depth as the volumetric water content (SWC) divided by the total porosity.

VI.2.4 Gas sampling at the soil surface

Soil CO_2 efflux was monitored in the +W and -W treatments using a dynamic closed-path Li8100 system equipped with a 20 cm diameter Li8100-103 respiration chamber (LiCor Inc., Lincoln, NE, USA). 54 PVC collars (9 per plot in treatments +W and -W and 3 blocks) were installed in May 2010 to sample different distances from the tree (Figure VI. 1), using the methodology described in Nouvellon et al. (2008). Soil CO_2 efflux was measured every 14 days over 3 years from December 2014 to December 2017, which represented 1.5 year before the clear-cut and 1.5 year after the clear-cut. In October 2016, 27 PVC collars were installed in the NH treatment to measure soil CO2 efflux from November 2016 to December 2017.

Soil CO_2, N_2O and CH_4 fluxes were measured simultaneously to the Li8100 measurements in the same plots. Chamber (diameter 40 cm, height 10 cm) collars were permanently set up in the +W, -W and NH plots (Metay et al., 2007). Four anchors were placed randomly in each plot at four distance from the trees, one month before the beginning of greenhouse gas efflux measurements (Figure VI. 1). Anchors were temporarily removed during stand harvest, then they were returned to the same location. Measurements were made every 14 days over one year from March 2015 to March 2016, then once a month between April 2016 and December 2017. Soil CO_2, N_2O and CH_4 fluxes were monitored over 3 months before the clear-cut and over 19 months after the clear-cut in the coppice-managed treatments +W and -W, as well as over 22 months in the NH treatment. The chamber atmosphere was sampled 4 consecutive times (0, 10 min, 20 min and 30 min after closure) during this period with 5 mL syringes previously purged.

Gas samples were analyzed using electron capture gas chromatography (CPG-ECD, at the Plant Production Department - FCA, UNESP, Botucatu, Brazil). Gas samples were analyzed in less than 36 hours after sampling (the validity of this period was verified in a preliminary study). Concentrations of CO_2, N_2O and CH_4 in soil air were calculated by comparison of integrated peak areas of samples with standard gases, which were used to make a four-point calibration. Calibration was done for each sampling date. More than 4000 gas samples have been analyzed in our study.

Gas fluxes at the soil surface were calculated from the following equation:

$$Fs = \frac{\Delta[Gas]}{\Delta t} \times \frac{1}{S} \times \frac{PV}{RT}$$

where Fs is gas flux (µmol m^{-2} s^{-1} or nmol m^{-2} s^{-1}), $\frac{\Delta[Gas]}{\Delta t}$ is the slope of the regression line of gas concentrations (ppm or ppb) over time (s), S is the chamber surface (m^2), V the chamber volume (m^3), P the atmospheric pressure (Pa), R the perfect gas constant (8.3143 J K^{-1} mol^{-1}) and T the temperature (K).

Cumulative soil CO_2, N_2O and CH_4 fluxes were estimated for each PVC collar and each chamber using linear interpolations of fluxes between each measurement date for all treatments. Gana et al. (2018) checked in a nearby eucalypt stand the validity of this method for CO_2 effluxes in comparison with monitoring every hour using automatic Li-cor chambers. Annual estimates of gas fluxes were made for each individual flux chamber.

VI.2.5 Gas sampling at different soil depths

The permanent pits in the +W and -W treatments were 1.5 m in diameter and reached a depth of 17 m (Figure VI. 1). They were maintained by concrete rings to reduce potential soil collapse and disturbances of the soil system. To prevent light and rain entering the pits, clear-colored roofs of the same diameter as the pits were used. Platforms were set up at two-meter intervals in the pits, equipped with artificial lighting and fans used during working sessions, allowing access and safe work down to the water table (Germon et al., 2019). The high cost of opening and securing these pits prevented further replications in the other two blocks. However, other measurements, including CO2, N2O and CH4 effluxes, were made in the three blocks, making it possible to extrapolate some of the observations made in the two deep pits. In October 2015, a third pit was excavated in the non-harvested treatment (NH) down to a depth of 3.5 m to study the clear-cut effect.

Soil CO_2, N_2O and CH_4 concentrations were measured at a depth of 0.1, 0.5, 1.0, 3.5, 7.5, 11.5 and 15.5 m (3 repetitions per depth, Figure VI. 1) in the +W and -W treatments and at 0.1, 0.5, 1.0 and 3.5 m depths (3 repetitions per depth) in the NH treatment. Soil-gas samples were collected using a passive method: a 20 mL syringe was driven into the ground at 1.2 m from the lateral pit wall for each sampling depth (to be sure to sample an undisturbed soil area) and remained permanently in the soil throughout the study period (Chapuis-Lardy et al., 2009; Metay et al., 2007). Additional 1 m deep pits (3 per treatment in +W, -W and NH) were excavated to collect soil-gas samples at depths of 0.1, 0.5 and 1 m to increase the number of replications in the top meter, and then backfilled with soil horizons in their correct order. Soil gas was pumped from the inside of the pit (for permanent deep pits) or from the soil surface into a 5 mL syringe (after several “micropurge” to prevent gas sample from contamination). This equipment was installed into the soil more than one month prior to the first soil gas measurement to allow for passive diffusion from the soil into the syringe. Soil CO_2, N_2O and CH_4 concentrations were measured on the same days as CO_2, N_2O and CH_4 effluxes at the soil surface from March 2016 to December 2017: over 3 months before clear-cutting and 19 months after the clear-cut in the coppice-managed +W and -W treatments and over 22 months in the NH treatment.

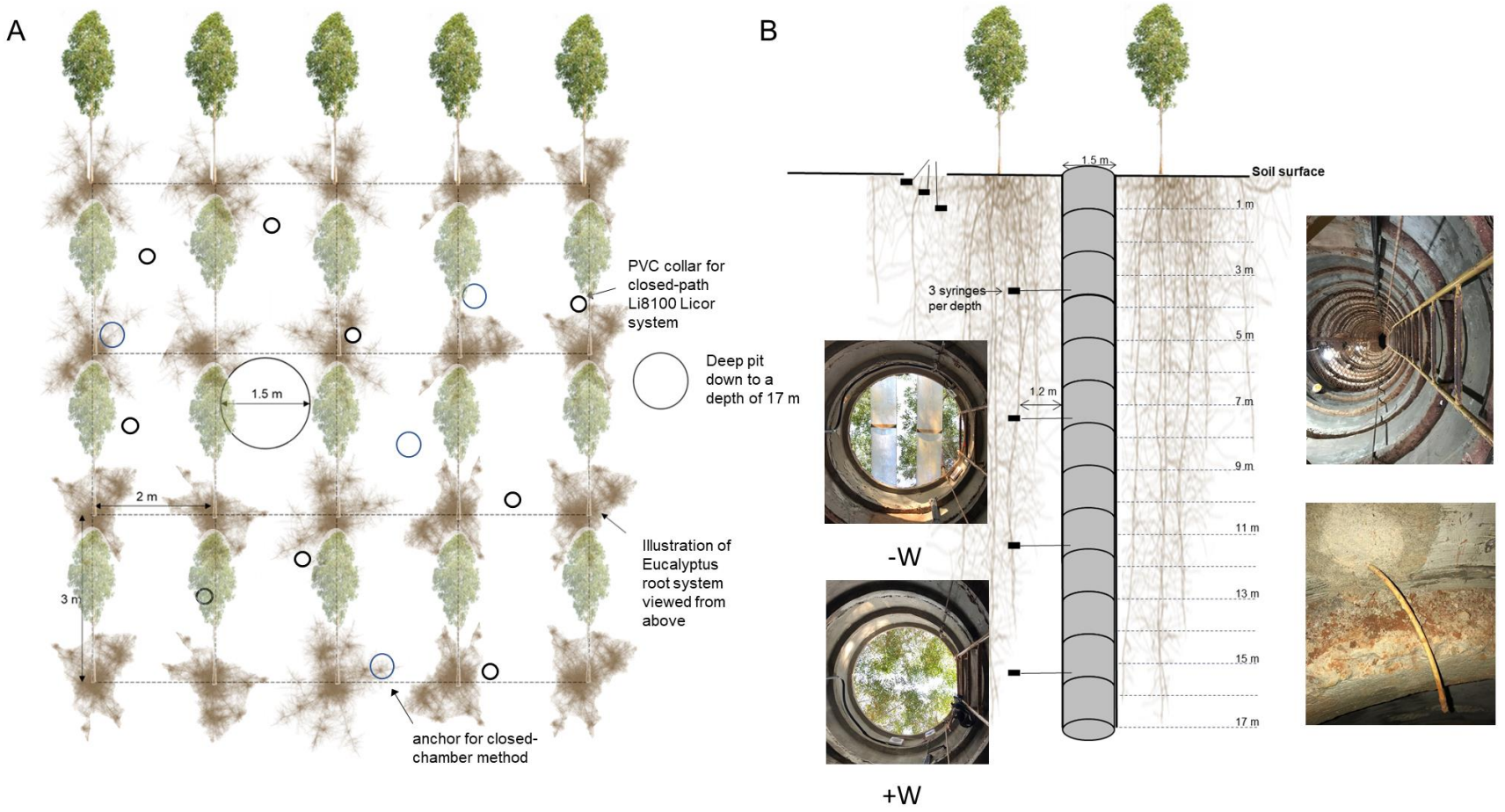


Figure VI. 1: A) Layout of the position of the pit in the +W treatment of block 1 and distribution of the PVC collars for closed-path Li8100 Licor system (n=9) and anchors for closed-chamber method (n=4). B) Layout of the position of the syringes for soil-gas sampling from the soil surface at 0.1, 0.5 and 1 m (n=3 per depth) and in the permanent pit at the depths of 3.5, 7.5, 11.5 and 15.5 m (n=3 per depth). The same set-up was used in the -W treatment. The photographs show a view from the bottom of each pit, the top of one pit and the tube used to sample soil gas.

VI.2.6 Modeling effective diffusivity of CO_2, N_2O and CH_4

Gas production at each depth was calculated as the difference between the flux across soil layers (Risk et al., 2002). To estimate soil gas fluxes within the soil profile we used CO_2, N_2O and CH_4 concentrations, soil physical properties and soil textural properties. Interlayer flux was determined from Fick's Lax in one dimension.

$$F(gas) = -Ds\ \frac{\delta C}{\delta z}$$

where Ds is the gas diffusivity in the soil, C is the concentration and z is the depth. This approach assumes that the concentration profile is momentarily at steady state. This model provides an estimate of the production that occurred below the depth of the calculated flux. The flux rates from soil gas concentrations depend on the soil gas diffusion coefficient. Effective diffusivity was estimated using the model of Moldrup et al. (1999), recommended for soils with clay contents < 30%:

$$\frac{Ds}{Do} = \frac{\varepsilon^{2.5}}{\sqrt{\varphi}}$$

where Do is the diffusivity of gas in air assumed to be 0.138, 0.144 and 0.195 $cm^2\ s^{-1}$ for CO_2, N_2O and CH_4, respectively, according to Massman (1998), ε is air-filled porosity and φ is the total porosity (Moldrup et al., 1999; Oerter et al., 2018). Air-filled porosity was calculated by difference between total porosity and soil volumetric water content at each depth.

VI.2.7 Statistical analyses

Linear mixed-effect models were used to test the effects of soil depth, treatment (+W and -W) and the interaction between depth and treatment on CO_2, N_2O and CH_4 concentrations in soil air, as well as CO_2, N_2O and CH_4 productions for the whole soil profile at each measurement date. Blocks were considered as random effects and residues were modelled by a first-order autoregressive correlation model to account for the correlations between soil depths. Two-way analyses of variance (ANOVAs) were used to assess the effect of treatments (+W, -W and NH) for individual soil layers on CO_2, N_2O and CH_4 soil concentrations. CO_2, N_2O and CH_4 fluxes at the soil surface were tested for statistical significance at each measurement date. The same analysis was made for cumulative CO_2, N_2O and CH_4 fluxes over the study period. The homogeneity of variances was verified, and log-transformation were used when the residuals

did not follow a normal distribution. Tuckey's post-hoc Honest Significant Difference (HSD) were used to determine the significant differences between treatments. Linear regressions were used to identify the correlation between CO_2 production and root biomass as well as soil CO_2 effluxes measured with the closed chamber method and the close-path Li8100 system. R software version 3.4.4 (Team R 2013) was used for all the calculations and statistical analyses with a level of significance of 5%. The lmerTest package was used to perform the linear mixed-effect models (Kuznetsova et al., 2017).

VI.3 Results

VI.3.1 Water filled pore space (WFPS)

WFPS values throughout the soil profiles were strongly influenced by throughfall exclusion and reflected the distribution of rainfall events (Figure VI. 2). Mean WFPS from March 2016 to December 2017 was 38.5 ± 12.7 % in the +W treatment and 31.7 ± 8.5 % in the -W treatment. At a depth of 0.5 m, WFPS ranged from 15.6% to 41.1% in the +W treatment and from 14.0% to 34.0% in the -W treatment. At a depth of 14 m, WFPS ranged from 34.7% to 53.7% in treatment +W and from 28.5% to 30.9% in treatment -W. At the end of the rainy season, mean WFPS in the 0-14 m soil layer were 37.9% and 30.7% in the +W and -W treatments, just before the clear-cut (June 2016), respectively, and 42.1% and 36.1%, 1 year after the clear-cut (June 2017). The soil profiles were refilled with water after the clear-cut as a result of an interruption in tree transpiration in coppice-managed stands. Water contents were lower in the -W treatment than in the +W treatment before the clear-cut and the difference between the two treatments persisted over the first 1.5 year after the clear-cut.

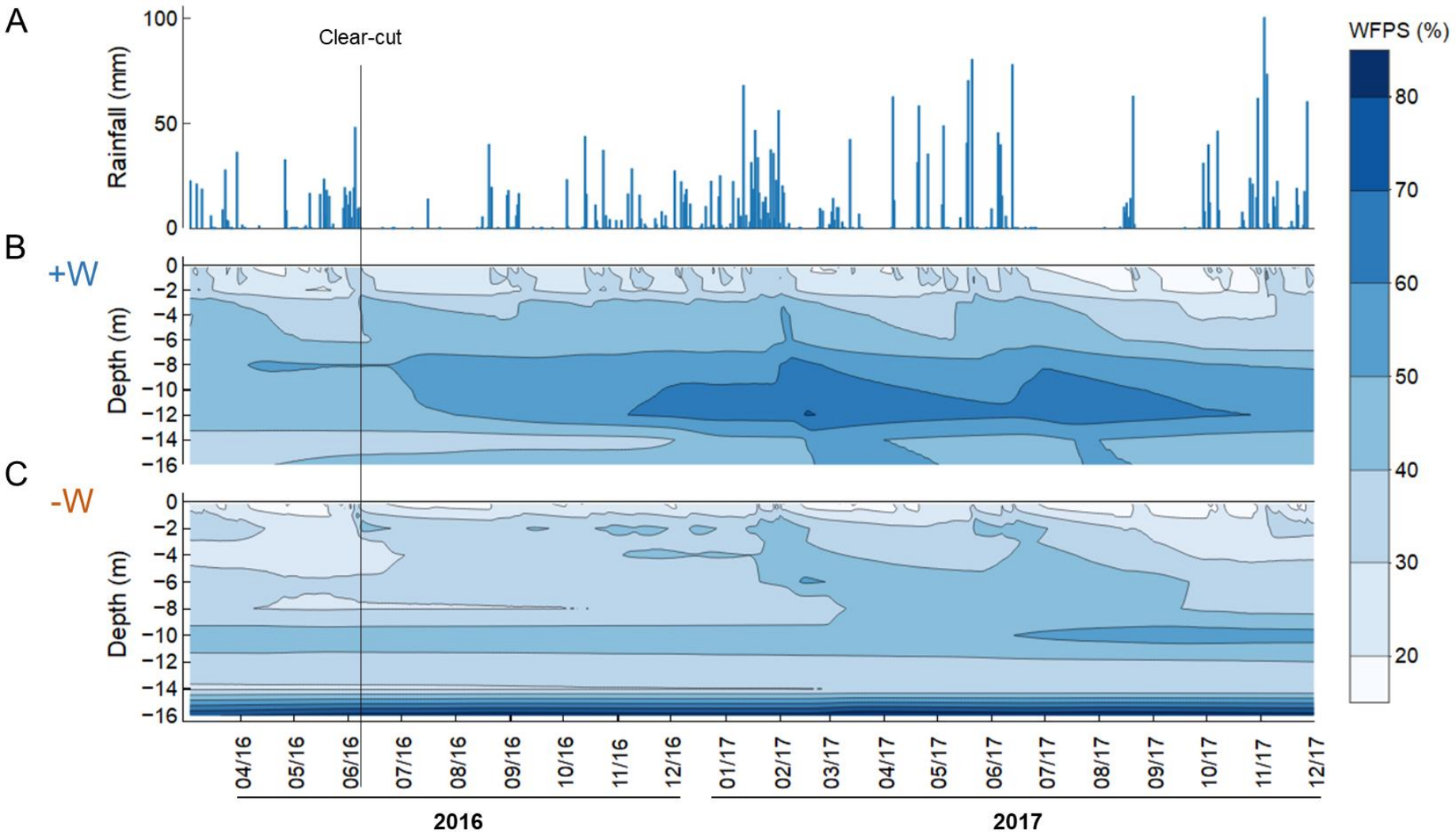

A
B
C
+W
-W
Clear-cut
Rainfall (mm)
100
50
0
Depth (m)
0
−2
−4
−6
−8
−10
−12
−14
−16
WFPS (%)
80
70
60
50
40
30
20
04/16
05/16
06/16
07/16
08/16
09/16
10/16
11/16
12/16
01/17
02/17
03/17
04/17
05/17
06/17
07/17
08/17
09/17
10/17
11/17
12/17
2016
2017

Figure VI. 2: Daily rainfall (A) and water filled pore space (WFPS, %) within the soil profiles down to a depth of 16 m in the undisturbed rainfall plots (+W, B) and in the plots with 37% of throughfall excluded by plastic sheets (-W, C) from March 2016 to December 2017. Water filled pore space graphical representation is a contour line interpolation obtained with marching square algorithm. R software version 3.4.4 and the “plotly” package version 4.8.0 were used. The clear-cut is indicated by a vertical line and the stand was coppice-managed after the clear-cut.

VI.3.2 CO_2, N_2O and CH_4 effluxes at the soil surface

Soil CO_2 effluxes measured with the Li-8100 device exhibited pronounced seasonal variations and reflected the dynamics of WFPS in the upper soil layers, with low values over the dry season (from June to September) and high values over the wet season (Figure VI. 3). Soil CO_2 effluxes over the study period ranged from 1.5 to 7.0 µmol m^{-2} s^{-1} in treatment -W and from 1.5 to 7.3 µmol m^{-2} s^{-1} in treatment +W. Cumulative surface effluxes the last year before the clear-cut were 136.5 ± 21.8 and 130.3 ± 24.8 mol m^{-2} $year^{-1}$ in treatments +W and -W, respectively, and they were 108.0 ± 15.8 and 119.3 ± 22.5 mol m^{-2} $year^{-1}$ the first year after the clear-cut in coppice-managed stands (Figure VI. 3). These cumulative soil CO_2 effluxes were not significantly different between treatments +W and -W. Soil CO_2 effluxes measured with the home-made closed-chamber system exhibited the same seasonal pattern and similar range of values as the soil CO_2 effluxes measured using the closed-path Li8100 system. The correlation between soil CO_2 effluxes measured from October 2016 to October 2017 by the two methods was high (r^2= 0.80), which suggests accurate estimates of N_2O and CH_4 fluxes using the closed-chamber method at our site (Appendix VI. 1). The clear-cut did not significantly (p = 0.379) affect soil CO_2 effluxes measured using the Li-8100 device (0.58 ± 0.19 and 0.47 ± 0.14 kg CO_2 m^{-2} $months^{-1}$ in NH and +W, respectively, from June 2016 to December 2017).

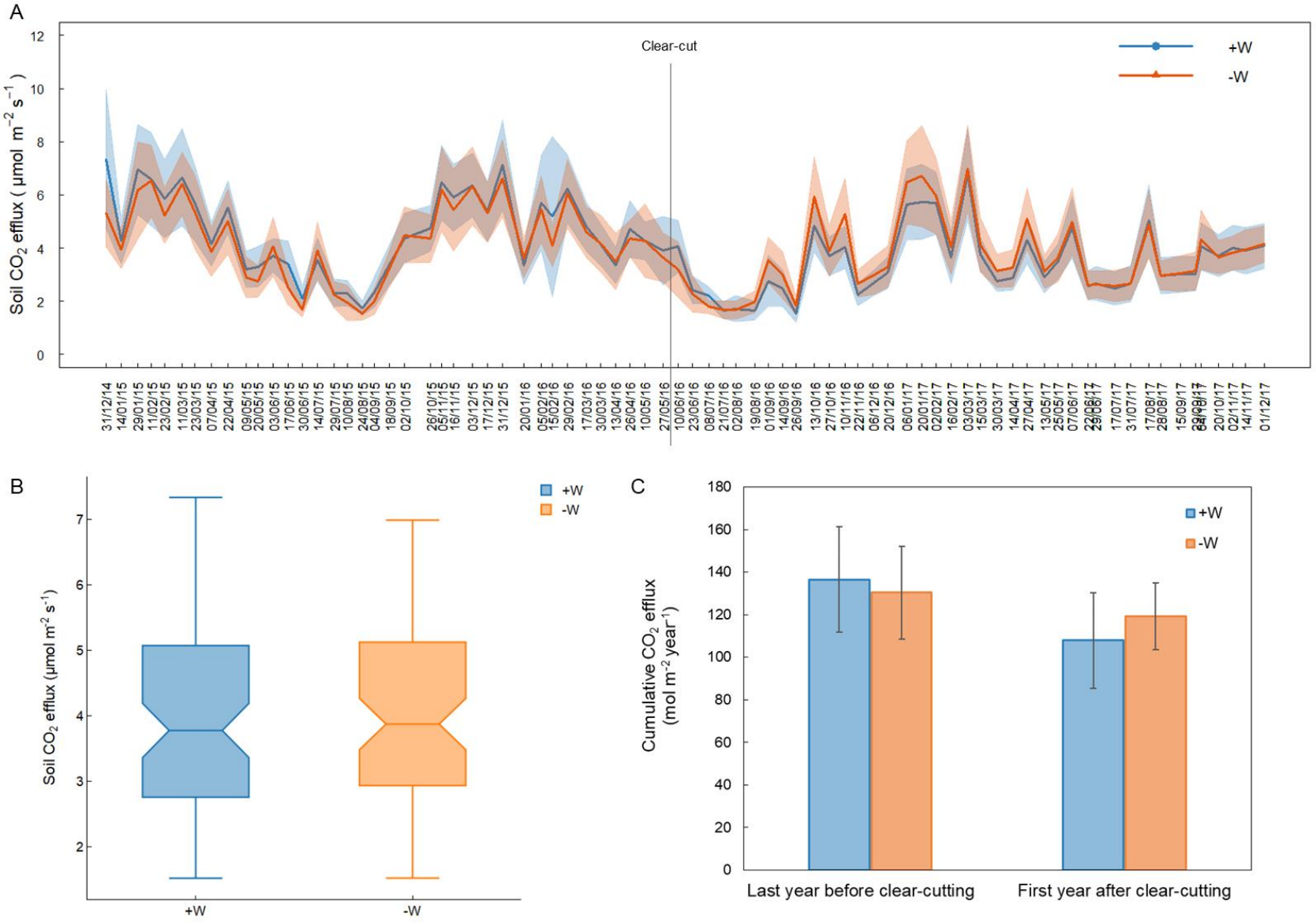
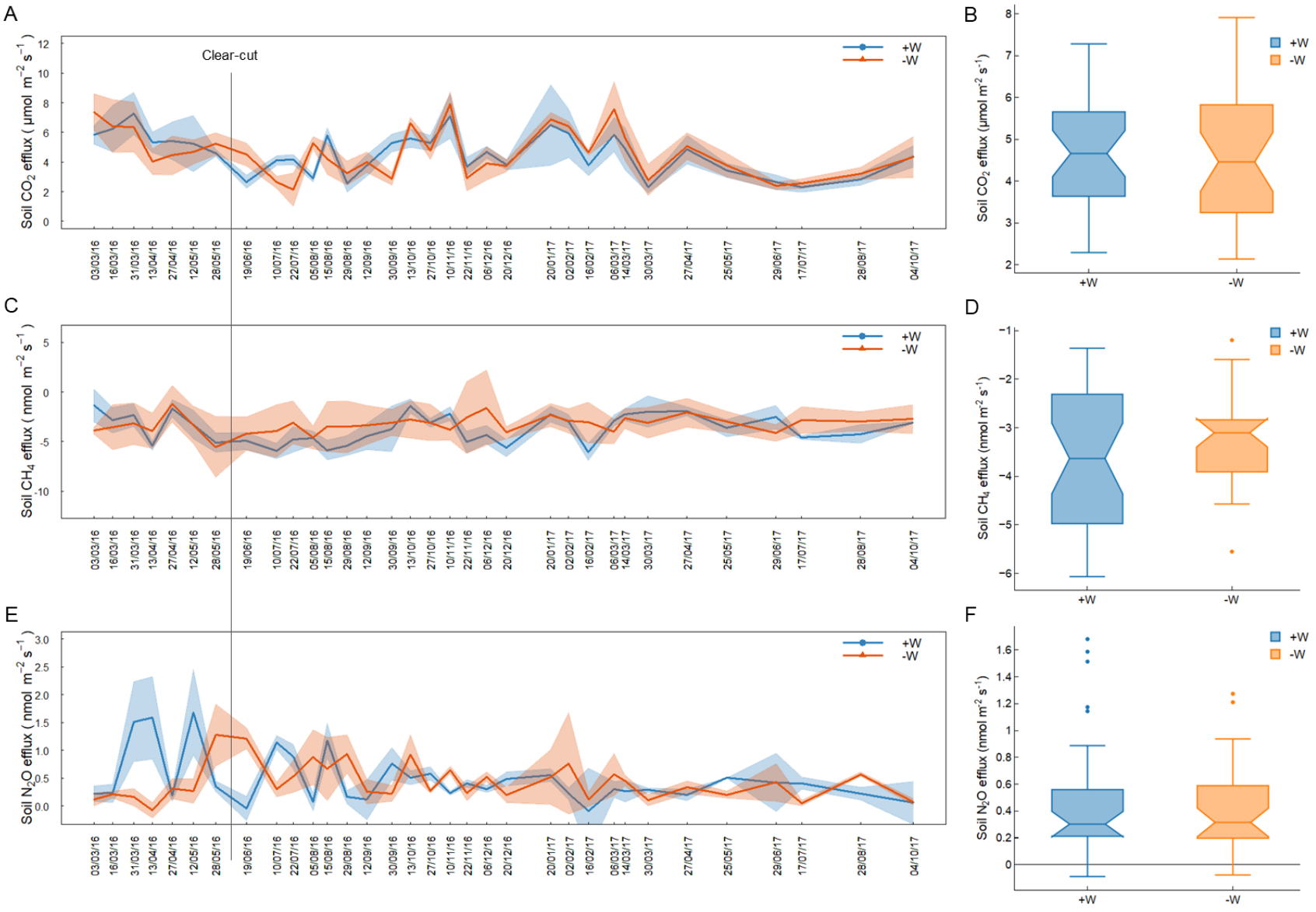

A
Clear-cut
+W
-W
Soil CO2 efflux (µmol m-2 s-1)
B
+W
-W
Soil CO2 efflux (µmol m-2 s-1)
C
+W
-W
Cumulative CO2 efflux (mol m-2 year-1)
Last year before clear-cutting
First year after clear-cutting

Figure VI. 3: A) Time course of soil CO_2 efflux (µmol m^{-2} s^{-1}) measured using the Li-8100 device in the undisturbed rainfall treatment (+W, blue) and in the treatment with 37% of throughfall excluded by plastic sheets (-W, orange) measured every 14 days from December 2014 to December 2017. Standard errors are shown (shaded area). The vertical line indicates the date of the clear-cut. B) Boxplots of soil CO_2 effluxes in treatments +W (blue) and -W (orange) from December 2014 to December 2017. Overlapping notches between boxplots suggest that the medians did not significantly differ. C) Cumulative soil CO_2 effluxes (mol m^{-2} $year^{-1}$) at yearly time scale in treatments +W (blue) and -W (orange), the last year before the clear-cut (between 5 and 6 years after planting) and the first year after the clear-cut in coppice-managed stands.

Soil CH_4 effluxes ranged from -6.1 to -1.4 nmol m^{-2} s^{-1} in treatment +W (-3.7 ± 1.5 nmol m^{-2} s^{-1} on average) and from -5.6 to -1.2 nmol m^{-2} s^{-1} in treatment -W (-3.3 ± 0.8 nmol m^{-2} s^{-1} on average) throughout the study period (Figure VI. 4). Negative effluxes of CH_4 at soil surface indicated a net consumption of atmospheric CH_4 by the soil. Similar cumulative soil CH_4 effluxes over 1.5 year after the clear-cut between treatments +W and -W (F = 4.002, P = 0.465), as well as between treatments +W and NH (F = 3.262, P = 0.116), showed that throughfall exclusion and clear-cutting did not significantly influence soil CH_4 effluxes (data not shown).

Soil N_2O effluxes ranged from -0.09 to 1.68 nmol m^{-2} s^{-1} in treatment +W (0.48 ± 0.46 nmol m^{-2} s^{-1} on average) and from -0.08 to 1.27 nmol m^{-2} s^{-1} in -W (0.43 ± 0.33 nmol m^{-2} s^{-1} on average)(Figure VI. 4). Similar cumulative soil N_2O effluxes over 1.5 year after the clear-cut between treatments +W and -W (F = 0.675, P = 0.412), as well as between treatments NH and +W (F = 2.748, P = 0.616), showed that throughfall exclusion and clear-cutting did not significantly influence soil N_2O effluxes (data not shown).


A
B
C
D
E
F
Clear-cut
+W
-W
Soil CO2 efflux (µmol m-2 s-1)
Soil CO2 efflux (µmol m-2 s-1)
Soil CH4 efflux (nmol m-2 s-1)
Soil CH4 efflux (nmol m-2 s-1)
Soil N2O efflux (nmol m-2 s-1)
Soil N2O efflux (nmol m-2 s-1)
03/03/16
16/03/16
31/03/16
13/04/16
27/04/16
12/05/16
28/05/16
19/06/16
10/07/16
22/07/16
05/08/16
15/08/16
29/08/16
12/09/16
30/09/16
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27/10/16
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22/11/16
06/12/16
20/12/16
20/01/17
02/02/17
16/02/17
06/03/17
14/03/17
30/03/17
27/04/17
25/05/17
29/06/17
17/07/17
28/08/17
04/10/17

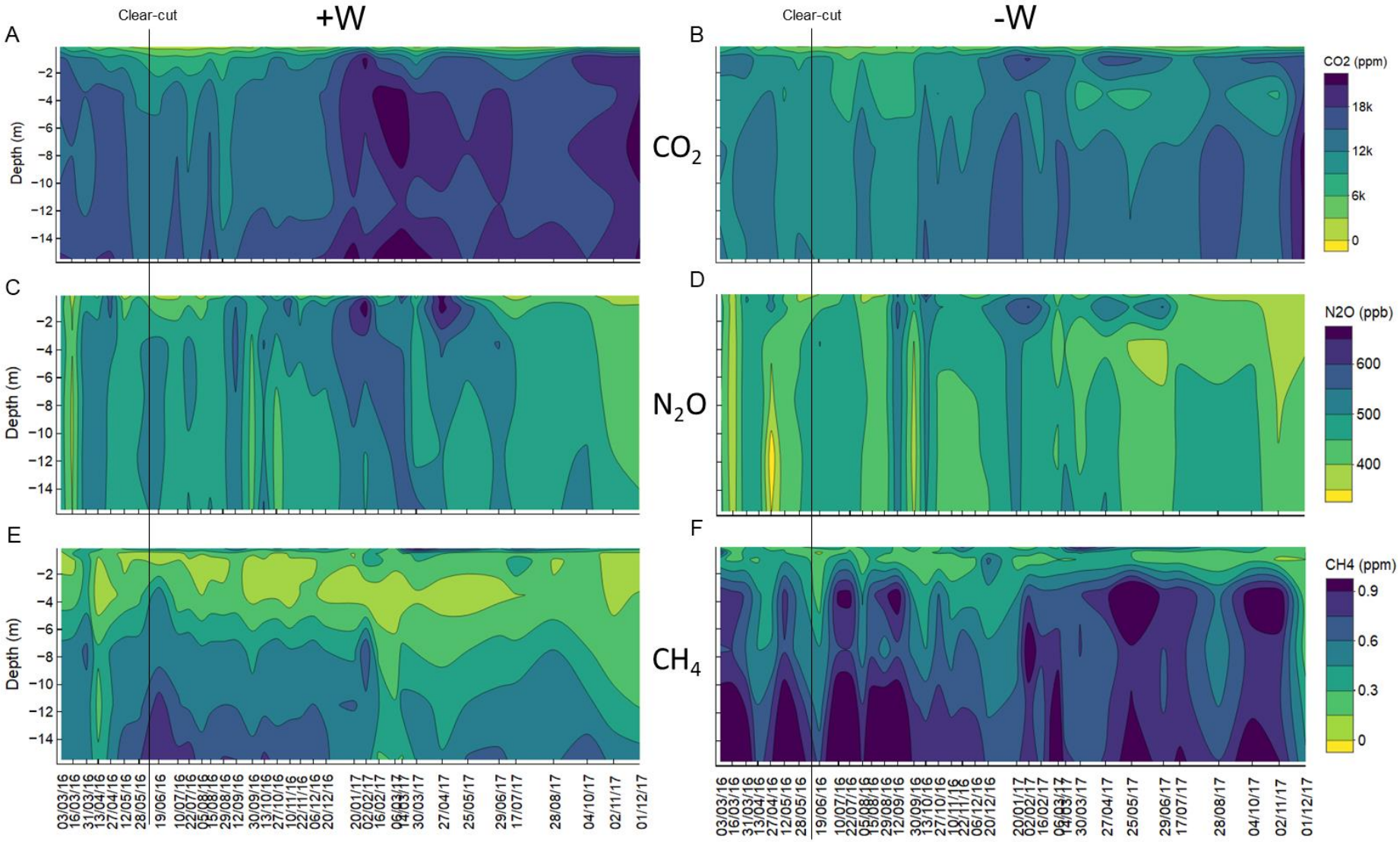
Figure VI. 4: Time course of soil CO_2 (µmol m^{-2} s^{-1}, A), CH_4 (nmol m^{-2} s^{-1}, C) and N_2O (nmol m^{-2} s^{-1}, E) effluxes in the undisturbed rainfall treatment (+W, blue) and in the treatment with 37% of throughfall excluded by plastic sheets (-W, orange) measured every 14 days from March 2016 to October 2017. The vertical line indicates the date of the clear-cut. Standard errors are shown (shaded area). Boxplots of soil CO_2 (B), CH_4 (D) and N_2O (F) effluxes from March 2016 to October 2017 in treatments +W and -W are shown. Overlapping notches between boxplots suggest that the medians did not significantly differ.

VI.3.3 Gas concentrations throughout deep soil profiles

Mean CO_2 concentrations from March 2016 to December 2017 were 22.1% higher in treatment +W than in treatment -W across the sampling depths (Figure VI. 5 A, B). CO_2 concentrations sharply increased with depth in treatments +W and -W. At a depth of 0.1 m, CO_2 concentrations ranged from 2438 to 9560 ppm in treatment -W (4774 ppm on average), and from 1700 to 9844 ppm in treatment +W (4178 ppm on average). At a depth of 15.5 m, CO_2 concentrations ranged from 9174 to 21784 ppm in treatment -W (13467 ppm on average), and from 13220 to 23938 ppm in treatment +W (18303 ppm on average).

Mean CH_4 concentrations from March 2016 to December 2017 were 37.5% lower in treatment +W than in treatment -W across the sampling depths (Figure VI. 5 C, D). CH_4 concentrations increased with depth in treatments +W and -W. At a depth of 0.1 m, CH_4 concentrations ranged from 0.14 to 0.92 ppm in treatment -W (0.37 ppm on average), and from 0.18 to 0.96 ppm in treatment +W (0.44 ppm on average). At a depth of 15.5 m, CH_4 concentrations ranged from 0.47 to 1.29 ppm in treatment -W (0.92 ppm on average), and from 0.27 to 0.89 ppm in treatment +W (0.61 ppm on average).

Mean N_2O concentrations from March 2016 to December 2017 were 7.6% higher in treatment +W than in treatment -W across the sampling depths (Figure VI. 5 E, F). At a depth of 0.1 m, N_2O concentrations ranged from 352 to 634 ppb in treatment -W (447 ppb on average), and from 355 to 685 ppb in treatment +W (454 ppb on average). At a depth of 15.5 m, N_2O concentrations ranged from 347 to 545 ppb in treatment -W (454 ppb on average), and from 400 to 573 ppb in treatment +W (496 ppb on average).


Clear-cut
+W
Clear-cut
-W
A
B
C
D
E
F
Depth (m)
−2
−4
−6
−8
−10
−12
−14
CO_2
N_2O
CH_4
CO2 (ppm)
18k
12k
6k
0
N2O (ppb)
600
500
400
CH4 (ppm)
0.9
0.6
0.3
0
03/03/16
16/03/16
31/03/16
13/04/16
27/04/16
12/05/16
28/05/16
19/06/16
10/07/16
22/07/16
05/08/16
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06/12/16
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20/01/17
02/02/17
16/02/17
30/03/17
27/04/17
25/05/17
29/06/17
17/07/17
28/08/17
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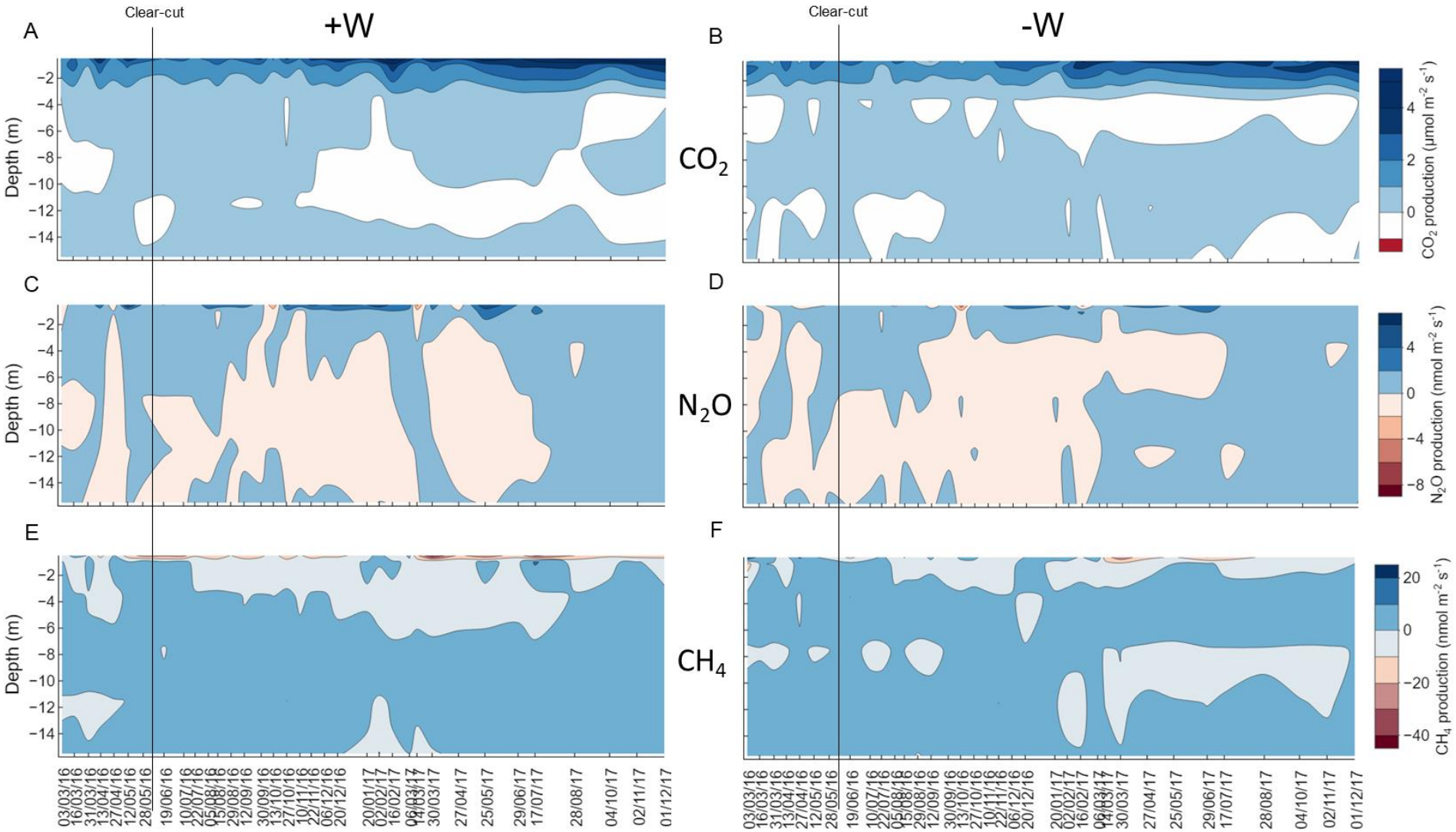
02/11/17
01/12/17

Figure VI. 5: Time course of CO_2 concentrations (ppm) within the soil profile down to a depth of 16 m (A) in the undisturbed rainfall treatment (+W) and (B) in the treatment with 37% of throughfall excluded by plastic sheets (-W), from March 2016 to December 2017. Time course of N_2O concentrations (ppb) within the soil profile down to a depth of 16 m in treatment +W (C) and in treatment -W (D). Time course of CH_4 concentrations (ppm) within the soil profile down to a depth of 16 m in treatment +W (E) and in treatment -W (F). Graphical representations are contour line interpolations using a marching square algorithm (R software version 3.4.4 and the "plotly" package version 4.8.0). The clear-cut date is indicated by a vertical line and the stand was coppice-managed after the clear-cut.

VI.3.4 CO_2, CH_4 and N_2O productions throughout soil profiles

CO_2, CH_4 and N_2O productions over the study period were much less influenced by throughfall exclusion than the concentrations throughout the soil profiles (Figure VI. 6). CO_2 and N_2O productions were highest and CH_4 production lowest in the upper soil layer (0-1 m), as a result of a large gradient of concentrations in the topsoil and high effective diffusivity. CO_2 and N_2O productions at depths of more than 4 m were close to 0 throughout the study period, and CH_4 productions were generally slightly positive in very deep soil layers (Figure VI. 6). At a depth of 0.1 m, CO_2 production ranged from 0.4 to 4.6 µmol m^{-2} s^{-1} in treatment -W and from 0.9 to 6.7 µmol m^{-2} s^{-1} in treatment +W, CH_4 production ranged from -26.4 to 21.3 nmol m^{-2} s^{-1} in treatment -W and from -41.1 to 5.2 nmol m^{-2} s^{-1} in treatment +W, and N_2O production ranged from -5.2 to 3.8 nmol m^{-2} s^{-1} in treatment -W and from -4.4 to 6.4 nmol m^{-2} s^{-1} in treatment +W. At a depth of 11.5 m, CO_2 production ranged from -0.06 to 0.08 µmol m^{-2} s^{-1} in -W and from -0.04 to 0.03 µmol m^{-2} s^{-1} in +W, CH_4 production ranged from -0.63 to 1.41 nmol m^{-2} s^{-1} in -W and from -0.13 to 0.41 nmol m^{-2} s^{-1} in +W and N_2O production ranged from -0.20 to 0.04 nmol m^{-2} s^{-1} in -W and from -0.04 to 0.04 nmol m^{-2} s^{-1} in +W. CO_2 production and fine root biomass (diameter < 2 mm) were strongly positively correlated across the soil layers (r^2= 0.76 for +W and r^2= 0.56 for -W) (Appendix VI. 2).

Whatever the soil layer, cumulated CO_2, CH_4 and N_2O fluxes over the first year after the clear-cut in coppice-managed stands were not significatively different in treatments +W and -W (Figure VI. 7). The cumulative CO_2 flux through the 0.1-0.5 m soil layer accounted for 76% and 53% of the cumulative CO_2 efflux at the soil surface in treatments +W and -W, respectively. The cumulative CH_4 flux through this soil layer accounted for 55% and 53% of the cumulative CH_4 efflux at the soil surface in treatments +W and -W, respectively. The cumulative N_2O flux through this soil layer accounted for 80% and 58% of the cumulative N_2O efflux at the soil surface in treatments +W and -W, respectively. The CO_2, CH_4 and N_2O fluxes through the soil layers dropped below a depth of 1 m. The small CO_2, CH_4 and N_2O productions in deep soil layers corresponded to high soil water contents (Figure VI. 8). The temporal variability of CO_2, CH_4 and N_2O production was high when the water filled pore space was below a threshold of 40%, with either CH_4 consumption or production in the upper soil layers depending on the sampling period.


A
+W
Clear-cut
B
-W
Clear-cut
CO2
C
D
N2O
E
F
CH4
Depth (m)
−2
−4
−6
−8
−10
−12
−14
CO2 production (µmol m-2 s-1)
4
2
0
N2O production (nmol m-2 s-1)
4
0
−4
−8
CH4 production (nmol m-2 s-1)
20
0
−20
−40
03/03/16
16/03/16
31/03/16
13/04/16
27/04/16
12/05/16
28/05/16
19/06/16
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01/12/17

Figure VI. 6: Time course of CO_2 production (µmol m^{-2} s^{-1}) calculated with the diffusivity model within the soil profile down to a depth of 16 m (A) in the undisturbed rainfall treatment (+W) and (B) in the treatment with 37% of throughfall excluded by plastic sheets (-W), from March 2016 to December 2017. Time course of N_2O production (nmol m^{-2} s^{-1}) within the soil profile down to a depth of 16 m in treatment +W (C) and in treatment -W (D). Time course of CH_4 production (nmol m^{-2} s^{-1}) within the soil profile down to a depth of 16 m in treatment +W (E) and in treatment -W (F). Graphical representations are contour line interpolations using a marching square algorithm (R software version 3.4.4 and the "plotly" package version 4.8.0). The clear-cut date is indicated by a vertical line and the stand was coppice-managed after the clear-cut.

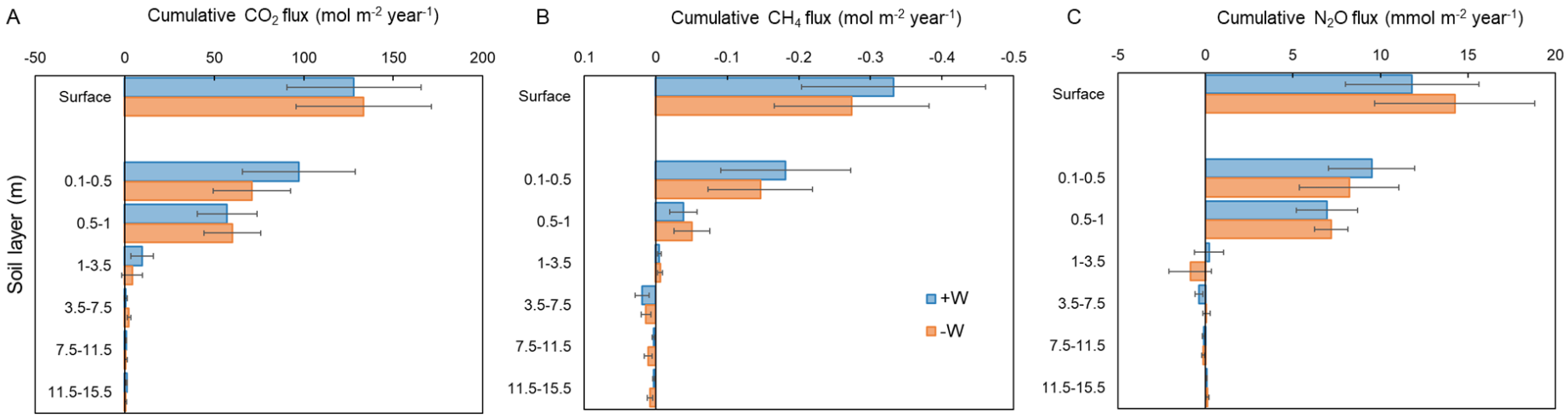

Figure VI. 7: A) Cumulative CO_2 fluxes (mol m^{-2} $year^{-1}$), B) cumulative CH_4 fluxes (mol m^{-2} $year^{-1}$), and C) cumulative N_2O fluxes (mmol m^{-2} $year^{-1}$) the first year after the clear-cut in coppice-managed stands in the undisturbed rainfall treatment (+W, blue) and in the treatment with 37% of throughfall excluded by plastic sheets (-W, orange). Cumulative fluxes of CO_2, CH_4 and N_2O were not significantly different ($P < 0.05$) between treatments +W and -W, whatever the soil layer.

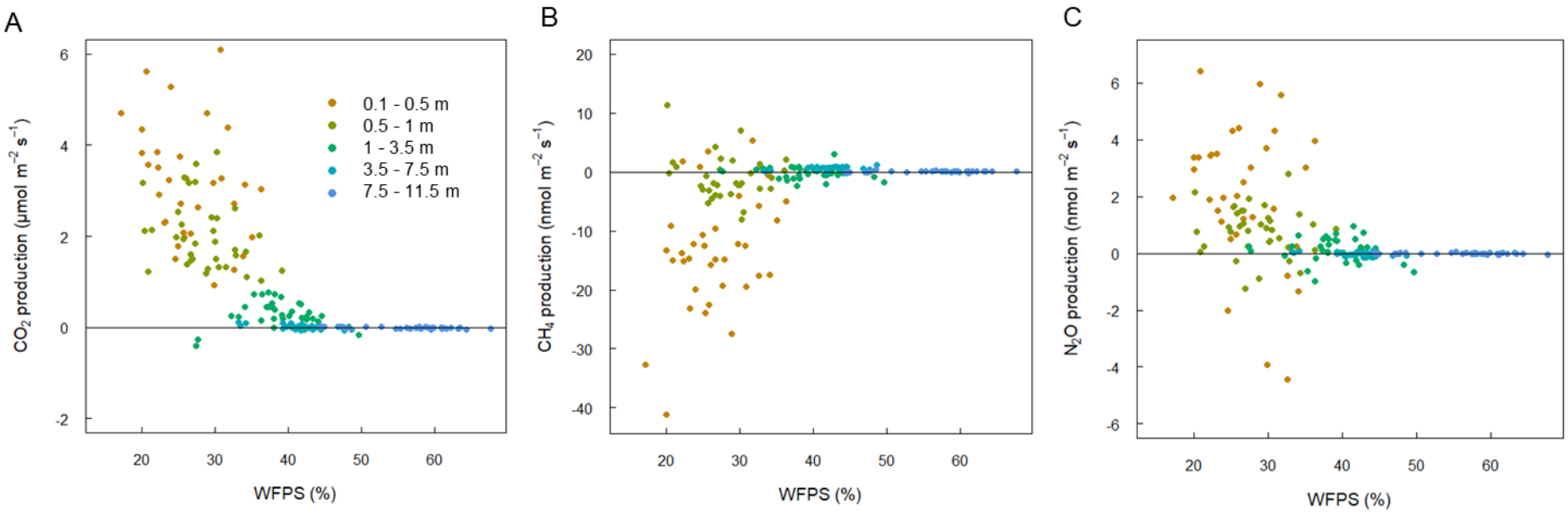

Figure VI. 8: Relationship between water filled pore space (WFPS) and CO_2 (A), CH_4 (B) and N_2O (C) production obtained using the diffusivity model for each soil layer 0.1-0.5 m, 0.5-1 m, 1-3.5 m, 3.5-7.5 m and 7.5-11.5 m in the undisturbed rainfall treatment +W.

VI.4 Discussion

VI.4.1 Effect of depth on soil CO_2, CH_4 and N_2O productions

The main novelty of our results lies in the first *in situ* measurements of CO_2, N_2O and CH_4 concentrations at depths of more than 10 m in tropical planted forests, which made it possible to estimate the dynamics of production of major greenhouse gases in response to contrasting management and environmental conditions. While CO_2 and CH_4 concentrations increased sharply with depth throughout the soil profile, N_2O concentrations where much less influenced by the depth. A strong increase in soil CO_2 concentration with depth has been reported in deep tropical soils with an order of magnitude consistent with our results. Soil CO_2 concentrations reached 80,000 ppm in a Brazilian Amazon rainforest (Davidson et al., 2004; Sotta et al., 2007; Trumbore et al., 1995), 30,000 ppm in a tropical rainforest in Costa Rica (Schwendenmann & Veldkamp, 2006) and 140,000 ppm in an agroforestry system in Indonesia (Van Straaten et al., 2010). The highest soil CO_2 concentration reached 25,000 ppm in our study at a depth of 15.5 m. Physical, chemical, mineralogical and biological properties can change considerably throughout deep soil profiles (Davidson et al., 2006; Fierer et al., 2003; Oerter et al., 2018; Wiaux et al., 2015). In our study, higher soil water contents in deep soil layers than in the topsoil led to a low proportion of air-filled pore space in deep soil layers and therefore a slow diffusion rate of the greenhouse gases to the atmosphere. *Eucalyptus* fine roots have been found down to the water table at a depth of 17 m in our experiment (Germon et al., 2019), and the deepest roots have an important functional role to withdraw water during dry periods (Christina et al., 2018). The sharp increase in CO_2 concentrations with soil depth might therefore reflect both CO_2 production in deep soil layers through root respiration and microbial decomposition of organic matter (dead roots, exudates, dissolved organic matter,…) and slow upward diffusion rates of gas in soil layers with a high proportion of water-filled pore space (Davidson et al., 2004; Schwendenmann & Veldkamp, 2006; Sotta et al., 2007). CO_2 production estimates from Fick's law highlighted that CO_2 production occurs mainly in the upper soil layers but small production rates occur down to a depth of 15 m. A low availability of microbial available C, very small nutrient concentrations in soil solutions and very low fine root densities in deep soil layers in our experiment (Germon et al., 2019; Laclau et al., 2010; Pradier, 2016) contributed to limiting the microbial activity (Fierer et al., 2003). The strong positive correlation between CO_2 production and fine root biomass along the soil profiles suggest that *Eucalyptus* roots are probably the main source of CO_2 in deep soil layers, both through root respiration and organic

inputs decomposed by microbes. The same pattern was shown down to a depth of 7 m in a moist tropical forest (Davidson et al., 2004). The seasonality of CO_2 production was stronger in the topsoil than in deep soil layers. CO_2 production was close to 0 above a threshold of water filled pore space of 45% and small negative values of CO_2 production were estimated in some deep soil layers. Negative values for CO_2 production in deep soil layers have been reported in other studies (Davidson et al., 2004; De Jong & Schappert, 1972; Risk et al., 2002). We speculate that high transpiration rates of *E. grandis* trees in our experiment (Christina et al., 2018) might lead to a withdrawal of dissolved CO_2 from deep soil layers to leaves through sap flow (Aubrey & Teskey, 2009; Bloemen et al., 2013; Ford et al., 2007; Hanson & Gunderson, 2009). Further studies are needed to quantify the flux of dissolved CO_2 via the xylem as *Eucalyptus* transform almost all incoming energy into latent heat flux through transpiration (Vezy et al., 2018).

CH_4 concentrations increase with soil depth in our study but they usually remain below the concentration in ambient air. CH_4 concentrations higher than in ambient air were measured at a few sampling dates in deep soil layers. As in our study, Davidson et al. (2004) found some soil areas with high CH_4 productions down to a depth of 11 m in a moist tropical forest. Spikes of CH4 concentration within the soil profile suggested that CH4 production occurred at various depths (down to 8 m) in a forest of the eastern Amazonia (Verchot et al., 2000). We show a CH_4 consumption in the topsoil and a weak CH_4 production in deep soil layers whatever the water supply regime in our experiment. CH_4 production primarily depends on the balance between two simultaneously occurring processes, production by methanogens and consumption by methanotrophic microorganisms (Dutaur & Verchot, 2007; Tate, 2015). Diffusion of gases are inversely proportional to the water-filled pore space. When the water-filled pore space decreases, the oxygen diffuses better and small changes in oxygen availability impact the balance between the two processes (Sanz-Cobena et al., 2014; Wang et al., 2018). Indeed, drier soil condition enhance CH_4 consumption whereas CH_4 production is favored by microsites deprived of O_2 in wet soil (Davidson, 1995).

Surprisingly, soil N_2O concentrations were little influenced by the depth in our eucalypt stands. By contrast, Verchot et al. (1999) showed a sharp gradient of N_2O concentrations down to a depth of 8 m in a tropical forest of the eastern Amazonia, which suggested a low production in deep soil layers. N_2O production in soils is driven by microbial nitrification (*i.e.* oxidation of NH_4^+ to NO_3^-) and denitrification (*i.e.* reduction of NO_3^- to N_2O) (Bai et al., 2014; Ortiz-Gonzalo et al., 2018; Zhong et al., 2014). Soil microbes consuming N_2O have been identified recently (Jones et al., 2013; Jones et al., 2014; Orellana et al., 2014; Sanford et al., 2012), and

the low changes in N_2O concentrations with depth in our study might result from a simultaneous production and consumption throughout the soil profile. The diffusivity model estimated slightly negative N_2O productions in some soil areas, which might a result of a reduction from dissolved N_2O to N_2 and/or a withdrawal from deep soil layers to leaves through sap flow (Chapuis-Lardy et al., 2007).

VI.4.2 Effect of throughfall exclusion on soil CO_2, CH_4 and N_2O productions

Contrary to our first hypothesis, throughfall exclusion did not significantly influence CO_2, N_2O and CH_4 effluxes at the soil surface as well as the production throughout the soil profile estimated with the diffusivity model. In both treatments +W and -W, soil CO_2 effluxes were within the range of measurements in tropical forest ecosystems (Cattânio et al., 2002; Davidson et al., 2004; Davidson et al., 2008; Nouvellon et al., 2012; Sotta et al., 2007). Higher CO_2 effluxes in wet season than in dry season suggest that soil moisture could be a strong driver of soil CO_2 emissions, as pointed out in other tropical and subtropical forests (Courtois et al., 2018; Davidson et al., 2010; Davidson et al., 2004; Sotta et al., 2007). Davidson et al. (2008) also found strong seasonal variations and no significant differences between control and throughfall exclusion treatments on CO_2 effluxes in an Amazonian forest. Unlike our observations, Sotta et al. (2007) showed in an Amazonian forest that CO_2 effluxes were lower in a throughfall exclusion plot than in the control plot during the dry season, and no differences were found between the control plot and the throughfall exclusion plot during the wet season. Fine root area index in treatment -W (32.8 m^2 m^{-2}) was significantly higher than in treatment +W (26.5 m^2 m^{-2}) in our 1.5-year-old coppice-managed *Eucalyptus* plantation (Germon et al., 2019), which may have offset the depressive effect of throughfall exclusion on microbial activity via a decrease in soil moisture.

In both treatments +W and -W, CH_4 effluxes at soil surface were strongly negative over the entire study period, which shows a net consumption of atmospheric CH_4 by the soil. CH_4 consumption is common in nearly all types of aerated soils: in forests (Davidson et al., 2004; Davidson et al., 2008; Nakano et al., 2004), deserts (Hou et al., 2012; McLain & Martens, 2006; Oerter et al., 2018), grasslands (Fang et al., 2014; Mosier et al., 1991) and tundra (Whalen & Reeburgh, 1990). A wide diversity of methanotrophs in tropical soils might account for steady CH4 effluxes at the soil surface in treatments +W and -W despite strong seasonal changes in soil water contents (Knief et al., 2005). N_2O effluxes at the soil surface were also similar in treatments +W and -W, with low seasonal variations. N_2O effluxes were generally lower in dry season than in wet season in an Amazonian forest, and throughfall exclusion decreased the

seasonal variability (Davidson et al., 2004). N_2O production is favored in wet soils while dry conditions favor the production of NO (Davidson et al., 2000a; Firestone & Davidson, 1989). Interruption of tree transpiration after cutting the trees in our experiment led to high soil water contents in the topsoil over most of the study period, which might explain the low influence of treatments +W and -W on greenhouse gas effluxes.

While CO_2, N_2O and CH_4 effluxes at the soil surface were not influenced by throughfall exclusion in our study, the concentrations of CO_2 and N_2O were 18.1 and 6.9% lower, respectively, and CH_4 concentrations were 60% higher in treatment -W than in treatment +W. Modeling effective diffusivity showed that the cumulated production rates of CO_2, N_2O and CH_4 in each soil layer were similar in the two treatments. The effects of throughfall exclusion on gas concentrations throughout the soil profiles were mainly a result of the influence of soil water contents on soil gas diffusivity. High soil water contents in the +W treatment lead to a saturation of the pore-space with water, which results in a slow diffusion of greenhouse gases to the atmosphere also pointed out in other studies (Maier et al., 2017; Wang et al., 2018). Throughfall exclusion experiments in tropical and subtropical forests show site-dependent responses to a decrease in throughfall for CO_2, N_2O and CH_4 concentrations in very deep soil profiles (4 m deep or more). Throughfall exclusion can either decrease (Cattânio et al., 2002) or increase (Davidson et al., 2004), N_2O concentrations in deep soil profiles and can lead to either similar (Davidson et al., 2004) or lower (Sotta et al., 2007; Van Straaten et al., 2011), CO_2 concentrations than in control plots. CH_4 concentrations in deep soil profiles were either similar (Davidson et al., 2004) or higher (Cattânio et al., 2002) in response to throughfall exclusion than in control plots.

VI.4.3 Effect of harvesting trees on soil CO_2, CH_4 and N_2O effluxes

Contrary to our third hypothesis, cutting all the trees did not lead to an increase in CO_2, CH_4 and N_2O effluxes, whatever the water supply regime. Harvesting *Eucalyptus* trees in plantations managed in short rotations lead to huge changes in soil environment likely to enhance organic matter decomposition, with an increase in soil temperature and soil moisture as well as accumulation of harvest residues at soil surface (Christina et al., 2017; Mendham et al., 2002; O'connell et al., 2004; Rocha et al., 2016). Another study in our experiment showed that harvesting did not lead to an increase in root mortality in the stands managed in coppice, whatever the water supply regime (Germon et al., 2019). We speculate that the lack of effect of clearcutting on CO_2 effluxes in our experiment may result from a decrease in root respiration after the harvest offsetting an increase in decomposition of organic matter at the soil surface.

Replanting present several drawbacks. Parfitt et al. (2001) showed that harvesting and replanting *Pinus radiata* resulted in a reduction of N status in soil, a loss of organic matter and an increase in soil respiration because roots from the previous rotation are decomposed and surface-soil microbial activity is enhanced following the clear-cut. Furthermore, harvesting and replanting are usually accompanied by considerable soil disturbance and also are longer exposed to wind and to erosion (Berhongaray et al., 2017). Coppice management might be an interesting option in *Eucalyptus* plantations managed in short rotation, to help mitigate the increase in CO_2, CH_4 and N_2O in the atmosphere and to limit soil erosion compared to systematic replanting.

Very deep tropical soils cover large areas worldwide and improving our understanding of soil-plant interactions throughout deep soil profiles is needed to improve the biogeochemical models used to predict the consequences of climate change on greenhouse gas effluxes in tropical planted forests. The dynamics of greenhouse gas concentrations in soil profiles result from the activity of microbial communities and can help gaining insight into the factors controlling biological activity in deep soil layers.

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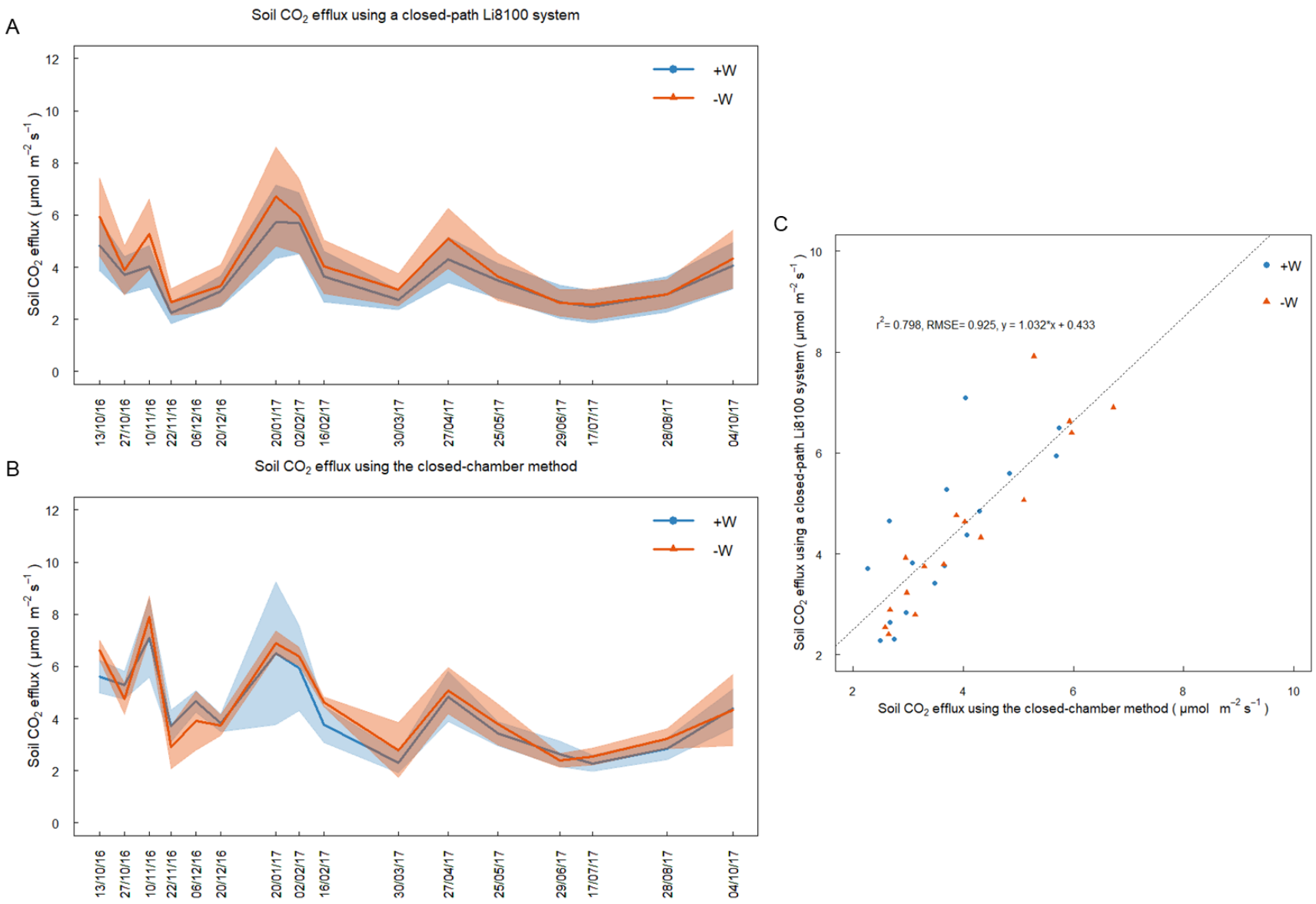
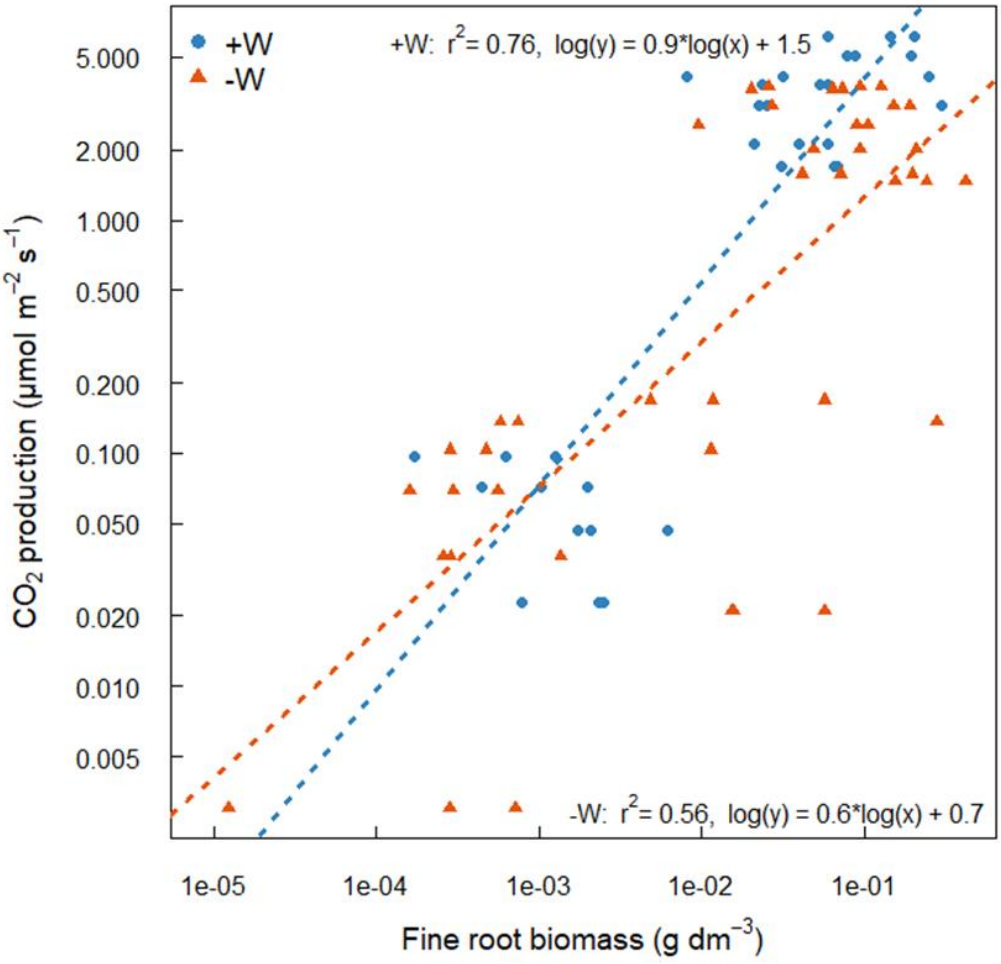
Appendix

Appendix VI. 1: Time course of soil CO_2 efflux (µmol m^{-2} s^{-1}) in the undisturbed rainfall treatment (+W, blue) and in the treatment with 37% of throughfall excluded by plastic sheets (-W, orange) measured every 14 days from October 2016 to October 2017 A) using the Li-8100 closed-path system and B) the closed-chamber method. Standard errors are shown (shaded area). Relationship between soil CO_2 efflux (µmol m^{-2} s^{-1}) measured using the Li-8100 closed-path system and the closed-chamber method in exclusion and undisturbed rainfall treatments (C).


A
Soil CO_2 efflux using a closed-path Li8100 system
Soil CO_2 efflux (µmol m^{-2} s^{-1})
+W
-W
13/10/16
27/10/16
10/11/16
22/11/16
06/12/16
20/12/16
20/01/17
02/02/17
16/02/17
30/03/17
27/04/17
25/05/17
29/06/17
17/07/17
28/08/17
04/10/17
B
Soil CO_2 efflux using the closed-chamber method
C
r^2= 0.798, RMSE= 0.925, y = 1.032*x + 0.433
Soil CO_2 efflux using a closed-path Li8100 system (µmol m^{-2} s^{-1})
Soil CO_2 efflux using the closed-chamber method (µmol m^{-2} s^{-1})

Appendix VI. 2: Relationship between fine root biomass (diameter < 2mm) and CO_2 production cumulated over 1 month (October 2017) in soil layers 0.1-0.5 m, 0.5-1 m, 1-3.5 m, 3.5-7.5 m and 7.5-11.5 m in the undisturbed rainfall treatment (+W, blue) and in the treatment with 37% of throughfall excluded by plastic sheets (-W, orange). Fine roots were sampled in October 2017.



What to remember from Chapter 6?

The main results of this chapter are summarized in the following Table VI. 1.

Table VI. 1: Main results obtained in the experimental set up on water filled pore space, CO_2, CH_4 and N_2O in deep soil layers, subjected to drought and in response of clear-cutting and coppice management. ↗ indicates a positive effect, ↘ indicates a negative trend and → indicates predominantly no effect.

Categories	**Sub-categories**	**Depth**	**Drought**	**Clear-cut**
Water	Water filled pore space (%)	↗	↘	↗
CO_2	Effluxes at soil surface		→	→
	Concentration in the soil	↗	↘	→
	Production within the soil profile	↘	→	→
N_2O	Effluxes at soil surface		→	→
	Concentration in the soil	→	↘	→
	Production within the soil profile	↘	→	→
CH_4	Effluxes at soil surface		→	→
	Concentration in the soil	↗	↗	→
	Production within the soil profile	→	→	→

CHAPTER 7: MODELING CO_2 PRODUCTION AND TRANSPORT IN A DEEP FERRALSOL UNDER EUCALYPTS PLANTATIONS SUBJECTED TO CONTRASTED RAINFALL REGIMES

The Chapter 7 is entitled « Modeling CO_2 production and transport in a deep Ferralsol under eucalypt plantations subjected to contrasted rainfall regimes ». The central objective of this chapter is to present the advances on a modeling approach carried out in this thesis in order to gain insight into the processes governing CO_2 transfer at a local scale. We aimed to couple a Numerical Model for the Analysis of the Geochemical Evolution of Mineral-Water-Air Systems (Min3P) with a simple root architecture model, Root-Typ.

To consider the distribution of eucalypt fine roots within the entire root profile, including the deep soil horizons, we first tried to calibrate the Root-Typ model (Part 1) with eucalypt root data measured at Itatinga, then we tried to include fine root dynamics in Min3P (Part 2).

This chapter will not be published in a scientific journal because the development initiated in this thesis could not lead to exploitable results.

However, the following co-authors are associated with and involved in the work: Loic Pagès, Christophe Jourdan, Jean-Paul Laclau and Céline Blitz-Frayret for the calibration and parametrization of the Root-Typ Model, and Frédéric Gerard, Guerric Le Maire, Jean-Paul Laclau and particularly Céline Blitz-Frayret for the calibration and parametrization of the Min3P model.

VII.1 Introduction

After a strong expansion in the last 20 years, forest plantations account for 7% of the total forest area in the world (Keenan *et al.*, 2015). In tropical and subtropical regions, plantations of fast-growing tree species store large amounts of carbon and contribute to reducing the exploitation of natural forests by providing an increasing proportion of the global demand of wood (Keenan *et al.*, 2015). Fast-growing *Eucalyptus* plantations cover about 20 million hectares throughout the world (Booth, 2013) with about 5.6 million hectares in Brazil (IBA, 2016). In Brazil, most of *Eucalyptus* plantations are settled in areas with very deep soils (> 5m) with low fertility and prolonged drought periods (Gonçalves *et al.*, 2013).

Future climate projections predict longer and stronger dry periods for eastern-south America (Solomon *et al.*, 2009). In this context, coppice management of eucalypt plantations could be an interesting option to face water deficit because, after the harvest, the trees are likely to benefit from an already installed root system making it possible to access to deep soil layers where water availability is higher than in the topsoil. Our aim was to gain insight into the consequences of drought on fine root production and mortality as well as on the production of greenhouse gases down to the water table (at a depth of 17 m). These effects have been monitored before and after harvesting in a coppice-managed *Eucalyptus grandis* stand under two contrasting water supply regimes: the undisturbed rainfall as a control and 37% throughfall reduction to assess the effects of water deficit on tree and soil functioning.

The consequences of tree harvesting on root dynamics the first year of the rotation in coppice-managed plantations ask specific questions never studied, particularly in deep soil layers. After the harvest, fine root mortality leads to an input of organic matter at different depths (Berhongaray *et al.*, 2015). Decomposition of fresh organic matter may enhance the degradation of pre-existing organic matter, stabilized or not (Fontaine *et al.*, 2007; Derrien *et al.*, 2014) through a "priming effect" (Kuzyakov *et al.*, 2000). The "priming effect" could affect carbon storage by the ecosystem and indirectly influence the nitrogen cycle. Most of the studies dealing with soil carbon storage are limited to the upper meter of the soil profile (Harper and Tibbett, 2013). The dynamics of CO_2 concentrations across soil profiles down to a depth of more than 10 m have never been studied in tropical forest plantations.

We have studied fine root dynamics (production and mortality) using the minirhizotrons technique and measured the concentrations of CO_2 down to the water table in the chapters 5 and 6. Root dynamics and gas concentrations were monitored before and after the harvest of

Eucalyptus grandis trees in a throughfall exclusion experiment, comparing plots with 37% of throughfall excluded by plastic sheets (-W), and plots without rain exclusion (+W). The main results obtained over 3 months before clear-cutting, then over 18 months in coppice, showed that CO_2 concentrations increased on average from 4475 ± 2180 ppm at a soil depth of 10 cm to 15885 ± 3538 ppm at a depth of 15.5 m across the two water supply regimes. CO_2 concentrations measured in treatment -W were on average 20.7% lower than in treatment +W, across the sampling depths.

We intended to go beyond the current measurements of gas concentrations at different depths in the soil profile through a mechanistic modeling approach making it possible to assess the production of gas throughout the soil profiles and to infer the sensibility of gas production to environmental factors. To better understand the dynamics of organic matter in deep tropical soils and to improve the modeling of element flows, it is necessary to quantify *in situ* the carbon (CO_2 as well as dissolved inorganic and organic C) concentrations throughout entire soil profiles (down to the root front), and to characterize the carbon stabilization/destabilization processes, as well as the main regulatory factors. A modeling approach was required to (i) quantify the production of CO_2 in different soil horizons from soil properties, root growth and gas concentration measurements throughout the profile, and (ii) to improve our understanding of transfer processes and the contribution of CO_2 deep production mechanisms to soil surface emissions (Maier and Schack-Kirchner, 2014).

Reactive transport models derived from Darcy's law allows a dynamic description of transient water flow in soils but also chemical processes controlling soil solution chemical composition (Feyen *et al.*, 1998; Simunek *et al.*, 2006; Samouëlian *et al.*, 2007; Steefel *et al.*, 2015). Reactive transport models require a vast number of input parameters and careful calibration procedures. Steefel *et al.* (2015) compared the key flow and transport features of the most commonly used reactive transport models: PHT3D, OpenGeoSys (OGS), ORCHESTRA, TOUGHREACT, eSTOMP, HYDROGEOCHEM, Crunch-Flow and MIN3P (Table VII. 1).

Table VII. 1: Comparison of the key flow and transport features of reactive transport models (adapted from Steefel *et al.* (2015)).

Capabilities/features	**PHT3D**	**OpenGeoSys**	**ORCHESTRA**	**TOUGHREACT**	**eSTOMP**	**HYDROGEOCHEM**	**CrunchFlow**	**MIN3P**
Dimensions	1,2,3 D	1,2,3 D	1D	1,2,3 D	1,2,3 D	1,2,3 D	1,2,3 D	1,2,3 D
Flow								
Saturated flow	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Richards equation	No	Yes	Yes	Yes	Yes	Yes	No	Yes
Multiphase-multicomponent flow	No	No	No	Yes	Yes	Yes	No	No
Variable density flow	No	Yes	No	Yes	Yes	Yes	Yes	Yes
Non-isothermal flow	No	Yes	No	Yes	Yes	Yes	No	Yes
Transport								
Advection	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Molecular diffusion	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Electrochemical diffusion	Yes	No	Yes	No	No	No	Yes	Yes
Dispersion tensor	Diagonal	Diagonal	Diagonal	No	Diagonal	Full	Diagonal	Diagonal
Gas phase advection	No	Yes	Yes	Yes	Yes	Yes	Yes	
Gas phase diffusion	No	Yes	Yes	Yes	Yes	Yes	Yes	

After a careful evaluation of the reactive transport models in the literature and their advantages and constraints to be adapted to the specificity of our study site (in particular very deep soil and monitoring over a long period), we selected the Min3P model already in use at the UMR Eco&Sols. This model was used in order to try to carry out simulations of the biogeochemical processes coupled with the transport of water, solutes and gases in soil (Mayer *et al.*, 2012). Indeed, gas transport is highly dependent on soil moisture and modeling soil water fluxes is therefore compulsory. The fate of organic matter in very deep soil horizons remains poorly documented, which is a serious concern of the current modeling approaches of soil carbon dynamics (Fontaine *et al.*, 2007; Rumpel and Kögel-Knabner, 2011; Mathieu *et al.*, 2015).

A modeling approach was needed to answer to the following questions:

- What are the main transport and reaction processes accounting for the observed CO_2 concentrations in a deep tropical soil explored by roots in eucalypt plantations?
- What is the contribution of fine roots in CO_2 effluxes at the soil surface?
- What are the consequences of drought periods on CO_2 production and efflux in eucalypt plantations established in deep Ferralsols?
- What are the consequences of future climatic scenarios on CO_2 production in deep soil layers and the effluxes at the soil surface?

Process-based models are needed to gain insight into the biogeochemical processes driving the concentrations of greenhouse gases measured at a few dates in soil profiles.

Modeling root architecture development is important to simulate temporal and spatial dynamics of the root system architecture. Root architectural models are also useful to estimate global root variables like biomass or surface over depth and time. They are parameterized by root type (e.g. RootTyp, Pagès *et al.* (2004); ArchiSimple, Pagès *et al.* (2014); DigR, Barczi *et al.* (2018)) or by branching order (e.g. OpenSimRoot; Postma *et al.* (2017); parametric L-system modeling; Leitner *et al.* (2010)). The number of parameters needed to simulate root architecture vary among models and infer their level of complexity (Dunbabin *et al.*, 2013). Root number, root diameter, root volume, and root radial distances are the main parameters of root system biomechanics (Nicoll and Ray, 1996; Danquechin Dorval *et al.*, 2016). RootTyp model showed promising efficiency in modeling root system development of *Quercu petraea* (Collet *et al.*, 2006) but also several species with various structures (Bingham and Wu, 2011; Chen *et al.*, 2011; Couvreur *et al.*, 2012). RootTyp model is easy to calibrate while combining many processes of root system development: (1) emission of new roots from the shoot system, (2)

elongation, (3) branching, (4) radial growth and (5) decay and self-pruning of existing roots. This architectural model can also be coupled with models simulating nutrient uptake (Mayer, 1999; Bonneu *et al.*, 2012; Mayer *et al.*, 2012), water transport (Doussan *et al.*, 1998) or rhizospheric processes (Kim and Silk, 1999) or even reactive transport model (Mayer, 1999; Mayer *et al.*, 2012).

We aimed to couple a Numerical Model for the Analysis of the Geochemical Evolution of Mineral-Water-Air Systems (Min3P) with a simple root architecture model, Root-Typ (Pagès *et al.*, 2004), to gain insight into the processes governing CO_2 transfer at a local scale. To consider the distribution of eucalypt fine roots within the entire root profile, including the deep soil horizons, we first tried to calibrate the Root-Typ model (Part 1) with eucalypt root data measured at Itatinga, then we tried to include fine root dynamics in Min3P (Part 2).

VII.2 Part 1: The Root Typ model

VII.2.1 Calibration and parameterization of the Root Typ model

Description and parameterization of the Root Typ model

The Root Typ model (Pagès *et al.*, 2004) simulates root growth processes at a daily time step: emission of new roots, elongation, branching and radial growth of various plant species by computing at each time step the root segments and the corresponding root length densities on a Cartesian grid. The model implements several developmental processes including root emission, axial and radial growth, sequential branching, reiteration, transition, decay and abscission. To simulate tree root systems, additional parameters are needed to simulate reiteration, senescence and late emission processes. All the parameters of Root Typ model are indexed to the apical diameter of all roots without distinction of root typology, which is impossible to assume in order to simulate growth and distribution of all types of roots of *Eucalyptus* trees within the soil profile. The issue was thus to adapt the Root Typ model to *Eucalyptus* trees.

Definition of root types

The Root Typ parametrization started with the definition of root types. In order to represent the most accurately *Eucalyptus* root systems we defined 6 root types. They are described and listed below (Figure VII. 1).

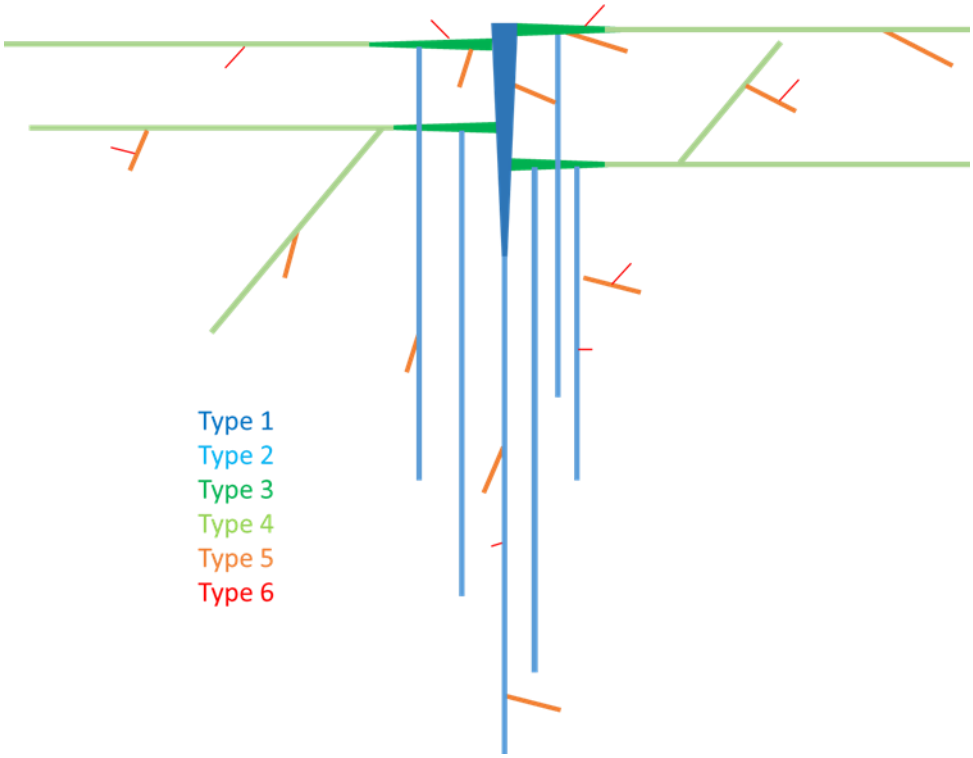


Figure VII. 1: Schematic representation of *Eucalyptus* root system in vertical view, with the different root types and their characteristics.

The root segments are not indexed to the position, so we had to create each time intermediate types for the macrostructure to distinguish between proximal and distal systems.

- Type 1: Juvenile tap root, orthotropic root from the radicle.
- Type 2: Mature tap root, orthotropic root that follow the juvenile tap root by transformation, can be carried by plagiotropic roots.
- Type 3: Juvenile plagiotropic macrorhize, plagiotropic root from the juvenile tap root.
- Type 4: Mature plagiotropic macrorhize, plagiotropic root that follow the juvenile plagiotropic roots by transformation.
- Type 5: Intermediate roots between macrorhizes and brachyrhizes
- Type 6: brachyrhizes. Extremely fine roots.

Parameters needed for modeling

For each root type, several root parameters are needed for this dynamic developmental model. There are listed below:

- Average and standard deviation of the insertion angle

- Duration before emergence
- Growth parameters: maximum, average and standard deviation of growth rate
- Inter-branch distance along the root
- Indicator of tropism type and tropism intensity
- Sensibility to mechanical constraint
- Apical diameter
- Necrosis duration
- Dates of reiterations and probability for reiteration at the reiteration dates
- Minimal and maximal number of reiterated roots
- Age from which transition may happen and daily probability of transition
- Proportions of type among the branches

A literature review made it possible to estimate all the parameters needed for the simulations. We mainly used data obtained in *Eucalyptus* plantations in Brazil and Congo. Most of the data dealing with root architecture came from the PhD thesis "Study of the root system of *Eucalyptus* in tropical plantation: architectural analysis, growth and respiration" of Armel Thongo M'Bou in Congo (Thongo M'Bou, 2008).
The next step was to include for each root type a coefficient for diameter increases throughout tree growth. Then we included into the code the possibility of choosing the seed to start the simulation. Those changes were important to represent accurately *Eucalyptus* root systems and to validate the output simulations made.

Setting up a R routine for calculation of root length per volume of soil

In order to represent an accurate root system, we had to estimate the root length simulated per volume of soil. A R routine was coded using the output of the Root Typ model to compare the results simulated with the data obtained in the field.

Modification of root density and ramification varying within the soil profile

We modified the soil profile component of the Root Typ model to include changes in root density and ramification within the soil profile. We also increased the soil profile depth down to 17 m.

VII.2.2 Main results obtained by simulations using the Root Typ model

We succeeded in simulating realistic root systems throughout the development of *Eucalyptus* trees (Figure VII. 2, Figure VII. 3, Figure VII. 5).

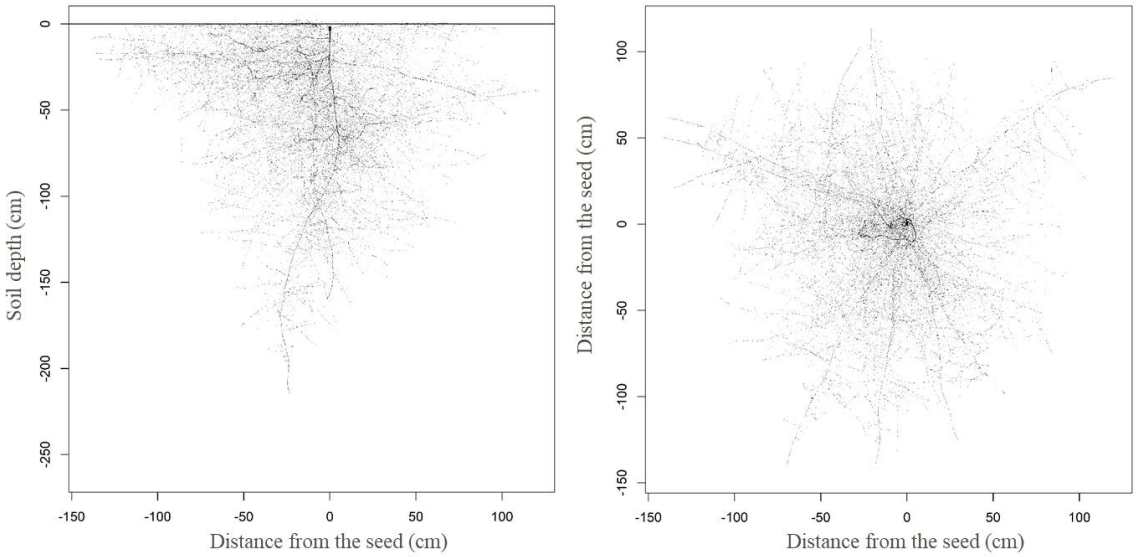
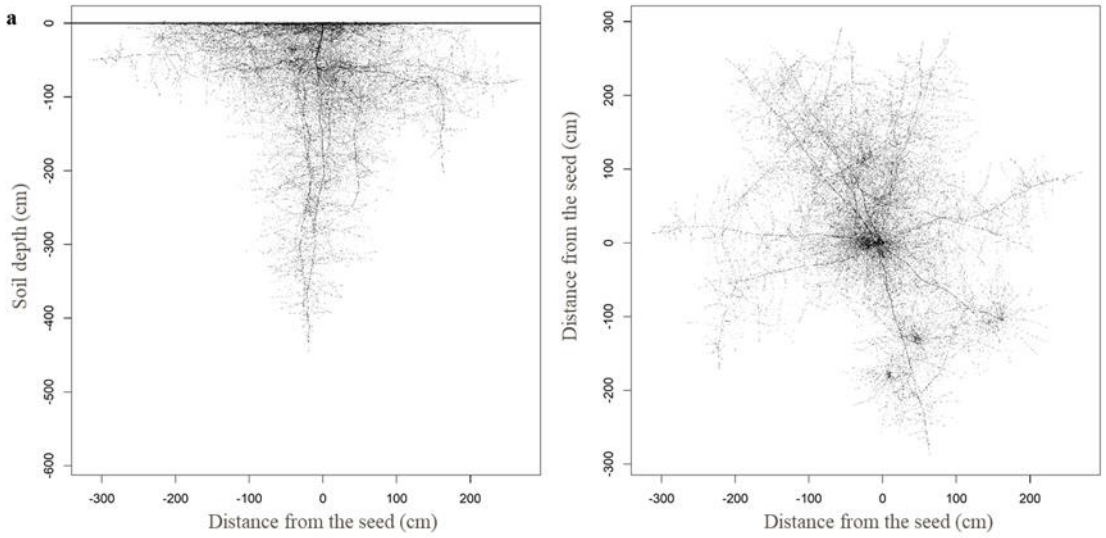


Figure VII. 2: Simulated *Eucalyptus* root system using the Root Typ model at age 6 months, in a XZ orientation and representation within the soil profile (left) and traced viewing from above the soil surface (XY) orientation (right).



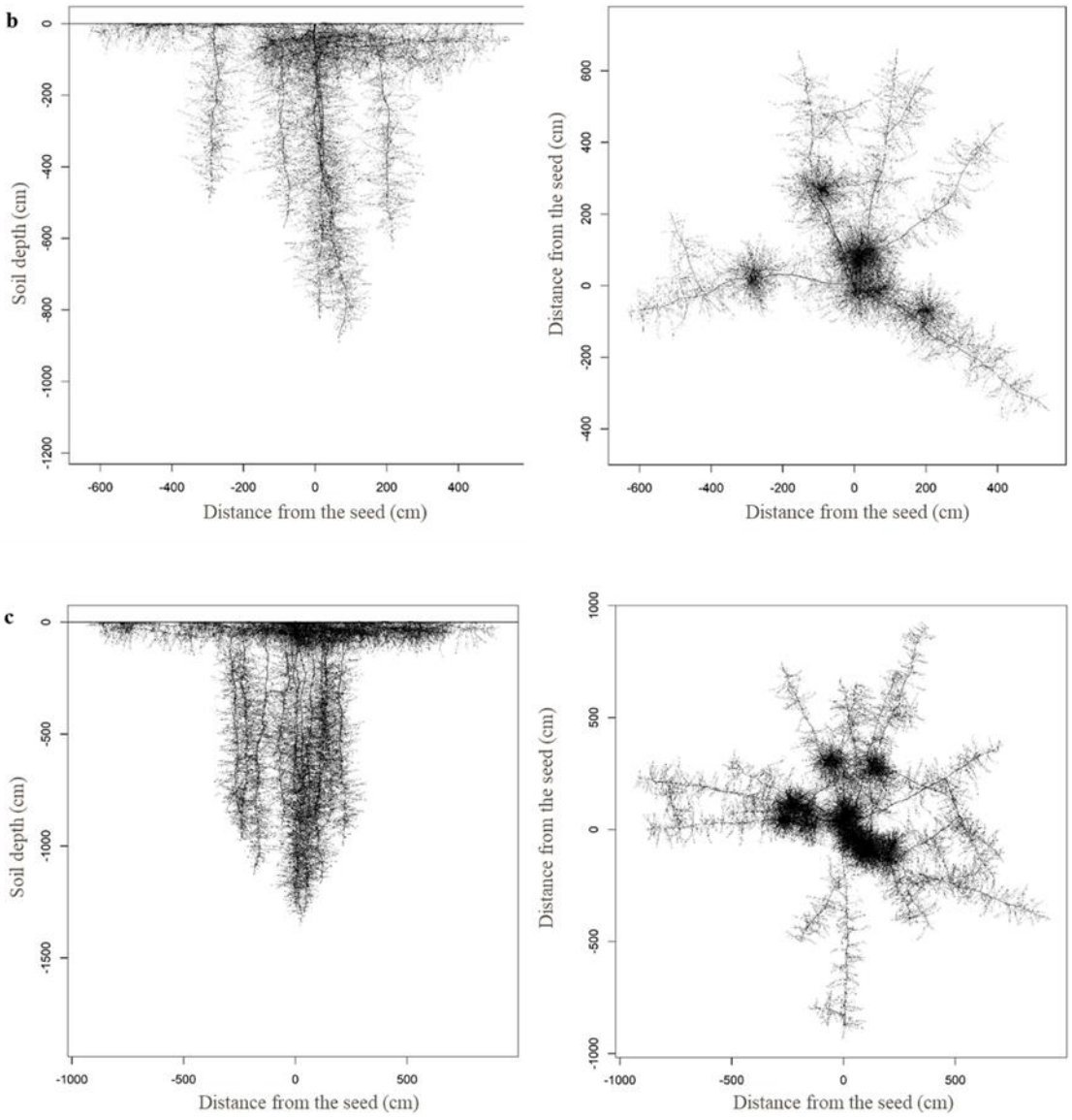


Figure VII. 3: Simulated *Eucalyptus* root system using the Root Typ model at age 1 year (a), 2 years (b) and 3 years (c), in a XZ orientation representation within the soil profile (left) and traced viewing from above the soil surface (XY).

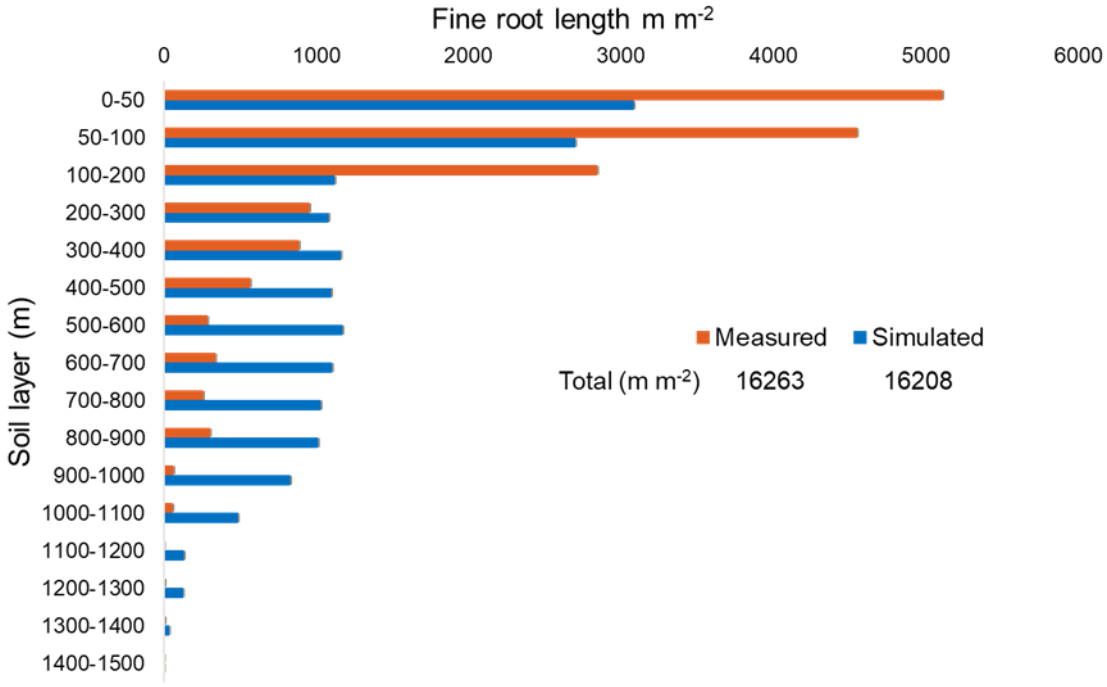


Figure VII. 4: Fine root length (m m^{-2}) per soil layer simulated using the Root Typ model (blue) and measured (collected in the field) for *Eucalyptus* plantations of age 3 years

Total root length obtained with the simulations using Root Typ Model were consistent with the measured data (observed in the field) for *Eucalyptus* plantations of 3 years of age, but the distribution throughout the different soil layers were not accurately simulated (Figure VII. 4).

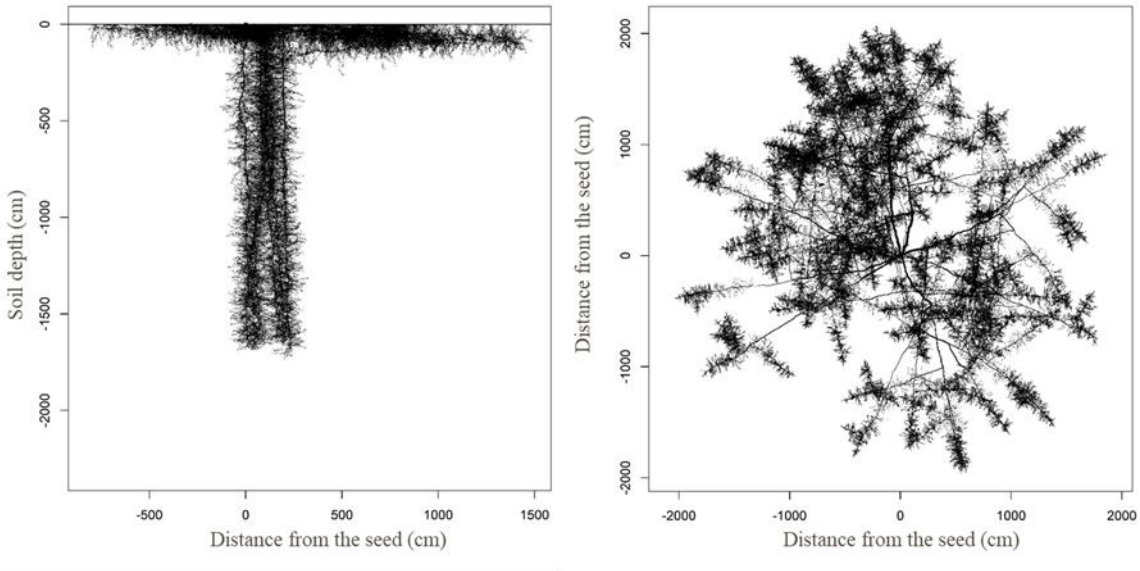


Figure VII. 5: Simulated *Eucalyptus* root system using the Root Typ model at age 6 years, in a XZ orientation and representation within the soil profile (left) and traced viewing from above the soil surface (XY) (right).

VII.2.3 Discussion and perspectives

Despite several attempts of simulations and 6 different modified versions of the Root Typ model, we did not succeed in designing simulated fine root densities close to the values measured in the field, especially between layers within the soil profile. Particularly, we did not manage to simulate the measured dynamics (too sharp mortality, high variability between the simulations). Therefore, we decided to force measured values as input values for root length density into the Min3P model in order to achieve the objectives of the project.

However, the improvement we made for the Root Typ model were important and more time is needed to parametrize and calibrate this model to tree root system development throughout an entire rotation. This work showed that the Root Typ model can be used to simulate tree root development over short period (up to 1-2 years) but more research is needed to simulate an entire rotation, as planned in our study. The two main issues rose in our project are listed and discussed here after.

Too much variability between seeds (simulations)

This is indeed a problem with stochastic models, when an event arriving early is very structuring for the future. In reality, compensating regulations operate, which are difficult to account for in this type of model. We should avoid in the setting up of the model, the very unlikely events that have a strong impact. For example, there must be a sufficient number of plagiotropic roots, varying their asymptotic lengths.

Exclusive pruning

All root branches have shorter root longevity than the bearing roots, therefore when branches are pruned the bearing axes become bare, which is never the case in the field. Therefore, a gap in the root length density was observed according to soil depth in the output simulations that we did not observe in field measurements within the entire soil profile.

Indeed, the fact of having a fixed lifespan for all roots belonging to the same diameter class generates unrealistic simulations. Several options could be tested in further studies, not necessarily exclusive:

- To create a root type that does not grow too fast with a wide range of length. Some will be pruned quickly and other should stay longer throughout the soil profile simulated

- To include into the code the possibility to have little branches (small branching densities) in such way that the longer roots carry some branches and therefore do not prune.
- To create a new root type of extremely fine roots that grow for longer period and branch or live longer than extremely fine roots that are pruned quickly.
- Include into the code a proximal reiteration regularly, to keep alive roots everywhere.
- Include into the code for a root lifespan different within the soil profile and for each root type.

Modeling architecture development and dynamics of deep fine roots are hindered by the limited data available in the literature yet indispensable to parametrize and evaluate the models. Root number, root diameter, root volume, and root radial distances are the main parameters of root system biomechanics (Nicoll and Ray, 1996; Danquechin Dorval *et al.*, 2016), varying considerably with soil layers. The plasticity of root system architecture development responding to their environment is strongly influenced by soil depth. Root architectural models through a simulation in 3D space of the root system could simulate accurately spatial and temporal dynamics. But integrate into a model a vertically discretized soils is particularly important as shallow and deep roots present different spatial and temporal dynamics. Model parametrization needs to consider shallow and deeper-rooted root system behaving differently to environmental factors and soil conditions.

VII.3 Part 2 : Min3P model

VII.3.1 Calibration and parameterization of the Min3P model

Description of Min3P model

Min3P (Mayer *et al.*, 2012) is a finite-volume reactive transport model including flow in the aqueous phase using Richard's equations and transport in the aqueous and gas phase. The flow and transport are computed on a representative domain of the field, composed of a Cartesian grid with elementary volumes called 'control volumes'. Primary variables of the model are aqueous concentrations, which are considered in equilibrium with gas-phase concentrations according to Henry's law. The use of the Dusty Gas Model for gas-phase transport allows the implementation of the transport mechanisms often neglected in previous studies (Molins *et al.*,

2008). The model formulation includes diffusion and advection to account for gas transport. Diffusion of gases is described by Stefan-Maxwell equations. To model diffusive fluxes with gases characterized by different molecular weights, a non-separative component may be present. Gas fluxes included in the experiment can be large enough to generate significant pressure gradients, leading to advective fluxes computed by Darcy's law. Field observations and laboratory studies have revealed that additional processes can control biogeochemical evolution in the vadose zone, possibly attenuating gas emissions in the atmosphere. For this reason, reactions taking place in the aqueous phase such as oxidation of methane by bacteria producing carbon dioxide, water and organic carbon, are also implemented. Finally, diffusive and advective gas fluxes and reaction source-sink term contribute to the mass balance.

Min3P is particularly suitable for the current study thanks to its implementation of the uptake of water and solute by plants (Gérard *et al.*, 2004; Gérard *et al.*, 2008). Indeed, gas transfer in the soil is highly dependent on soil water content and modeling soil water fluxes is therefore compulsory (Figure VII. 6). Separate sink terms have been implemented in the Richard's equation to account for physical evaporation and transpiration (components of the root water uptake) in Min3P. For each control volume of the grid, the physical evaporation is depending on the local water saturation and is considered as a one-dimensional process in vertical direction along a soil column. To model the biological plant transpiration process, the evaporative budget must be estimated and is determined with the soil moisture, allowing the consideration of the negative effects of water stress on plant transpiration. Finally, the solute uptake has been implemented by an additional sink term in the mass balance equation. Then, two uptake regimes are considered: 1) passive solute uptake where the solute uptake rate is equal to the water uptake rate and 2) rejective or active solute uptake. Rejective uptake implies the solute to be left behind water during uptake by the root (leading to an increase of solute quantity in the root zone) and active uptake implies a preferential solute uptake by the plant root (leading to a solute depletion in the root zone).

The first key step was to calibrate and parameterize the water transport. The simulations of water dynamics were validated using measurements of: (i) actual transpiration fluxes and (ii) volumetric soil water content monitored over the whole study period. The climatic variables (potential and actual transpiration fluxes, precipitation) determined at the experimental site were used either to constrain the model or to validate it.

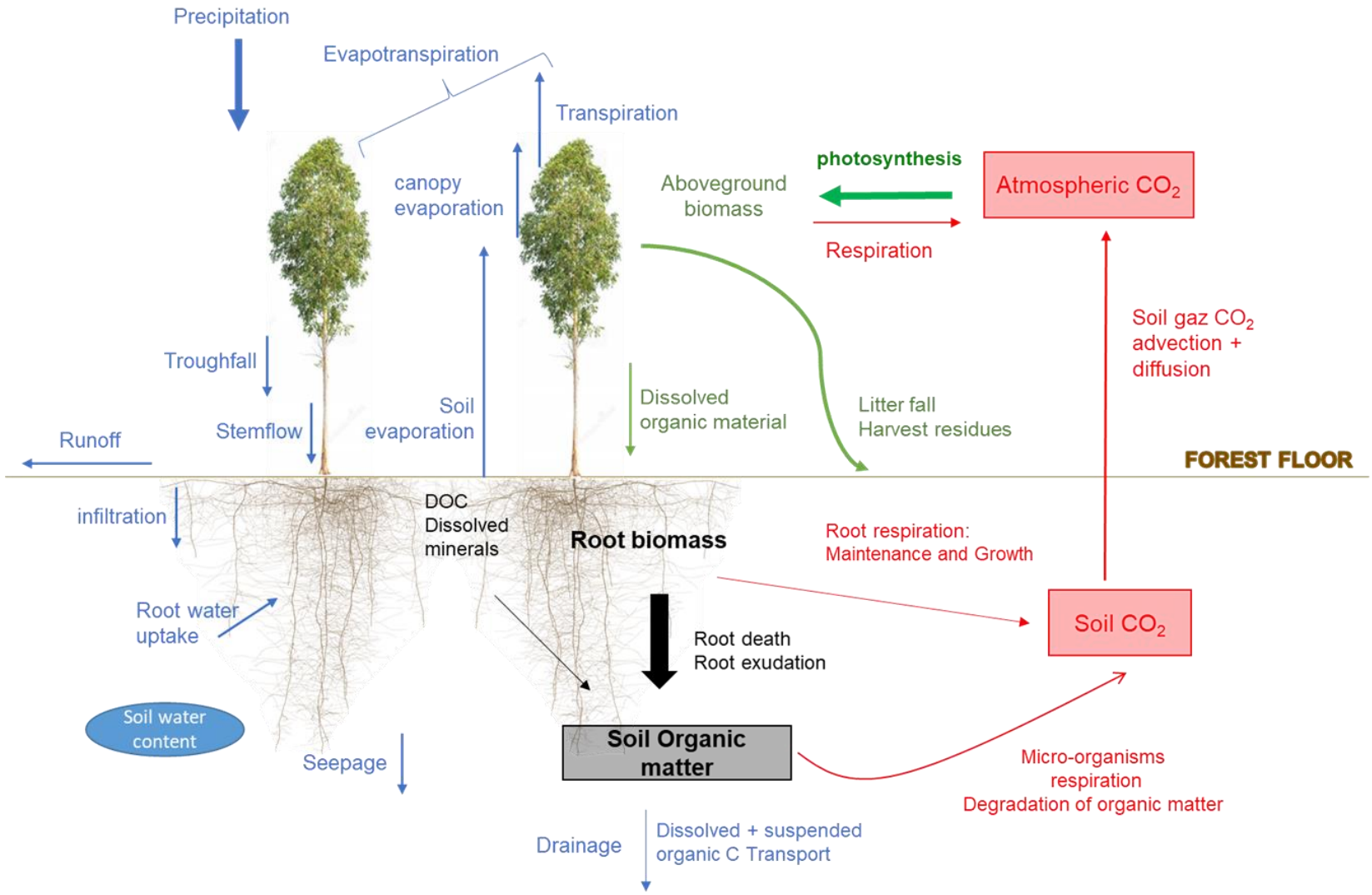


Figure VII. 6: Graphical representation of the close link between water transport and CO_2 production, transport and effluxes.

Soil parameterization and root profiles

Three layers of 33.3 cm thickness were defined to account for the variability of soil and root characteristics in the upper meter. Layers of 50 cm were defined from a depth of 1m to a depth of 18 m. Soil retention curves and hydraulic conductivity were estimated at the same site using Van Genuchten equations. In each soil layer, the soil water potential was linked to the soil water content by the relation of Van Genuchten (1980).

$$Sa = Sra + \frac{1 - Sra}{(1 + |\alpha \Psi a|^n)^m}$$

$$m = 1 - \frac{1}{n} \qquad Sa = \frac{\Theta a}{\Theta sa} \qquad Sra = \frac{\Theta ra}{\Theta sa}$$

With:

Sa saturation of the aqueous phase

Sra residual saturation of the aqueous phase

Θa volumetric water contents

Θsa volumetric water content at saturation

Θra residual water content

Ψa pressure head

Residual soil water content in each soil layer was calibrated using MAESPA model (Christina *et al.*, 2017) used at the same site. The MAESPA model, fully described in Duursma and Medlyn (2012), is a soil-plant-atmosphere model simulating forest canopy radiation absorption, photosynthesis and water balance. Soil hydraulic conductivity at saturation was measured at the same site down to a depth of 3 m (Maquere, 2008) and assumed to decrease linearly from 3 to 7 m depth and to be constant for the soil layers below 7 m down to 18m. Soil porosity was also measured down to 3 m and assumed constant below 3m. Evapotranspiration and soil evaporation were obtained by simulations using MAESPA model each day over the entire rotation (6 years).

A literature review and simulations of the MAESPA model were realized to obtain the initial condition variability-saturated flow. The initial condition of hydraulic charge, pressure head, saturation, soil water content and root evaporation are necessary for flow simulations. Other inputs needed for the simulation are the daily climatic data.

We were not able to use the outputs of the Root Typ model, so we estimated a relation between root length density, depth and time using root distributions along the soil profile measured at 1, 2, 3 and 7 years after planting. Root distributions were measured by soil coring (see Germon *et al.* (2018) and Christina *et al.* (2011) and Chapter 5 for the method).

$$RLD_{Sim} = \exp(-12.48 + 1.725 * \sqrt{Depth} + 0.0017 * time + 0.003961 * \sqrt{Depth * time})$$

With:

RLD_{Sim}, Simulated root length density

Depth, Soil depth in cm

Time, time in days

This equation was added into the Min3P code and we also modified the code to print soil water content outputs every day along the simulation time.

VII.3.2 Discussion and perspectives from simulations using Min3P model

The simulations results in Figure VII. 7 show that the soil water contents can be modeled over an entire rotation, but improvements are still needed. Unfortunately, we did not have enough time to conclude the parameterization and calibration of the Min3P model.

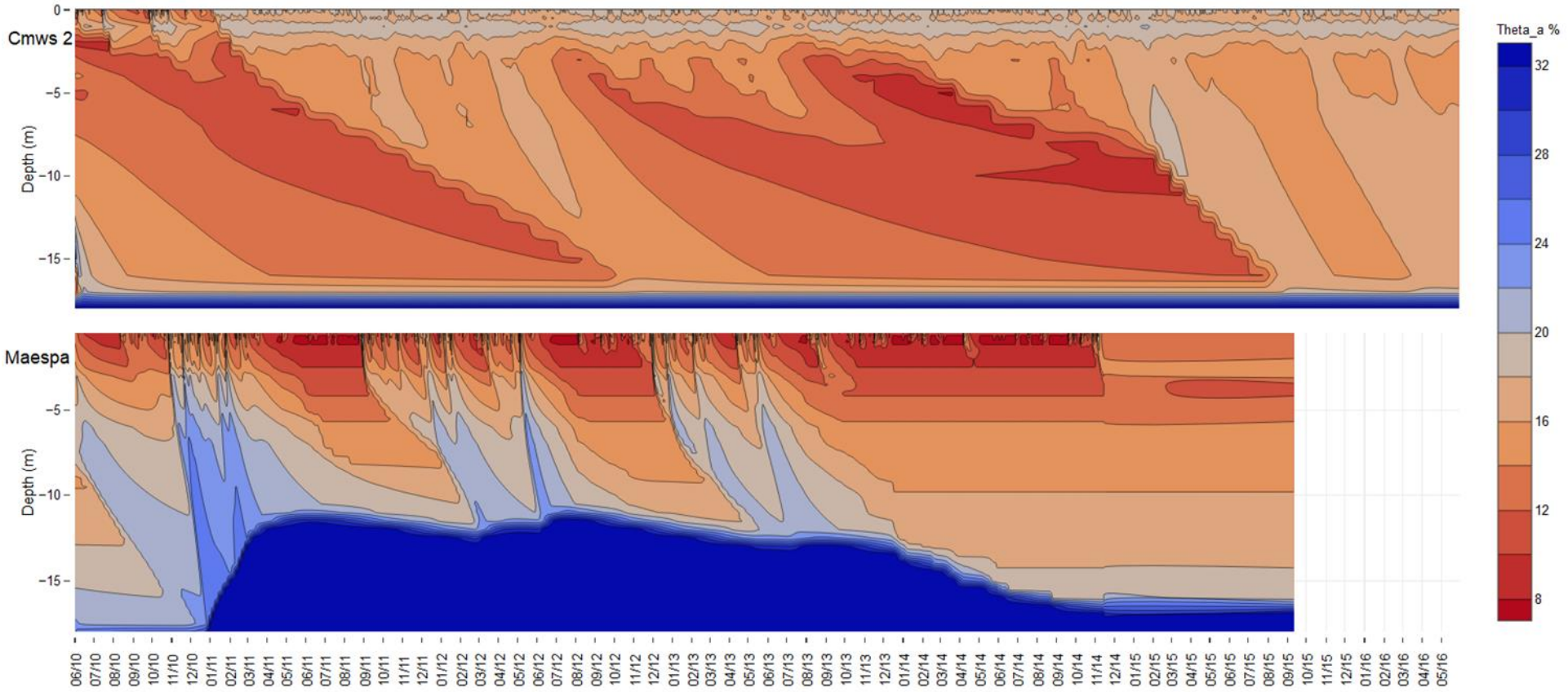


Figure VII. 7: Soil water contents (Theta_a %) within the soil profile down to a depth of 18 m simulated with the Min3P model (Cmvs 2, top) and simulated with the MAESPA model (Maespa, bottom) every day from June 1st, 2010 to May 31, 2016, which was the entire rotation for this *Eucalyptus grandis* plantation in the undisturbed rainfall plot. Soil water content graphical representation is a contour line interpolation using marching square algorithm. R software version 3.4.4 and the package plotly version 4.8.0 were used.

Model parameterization should be better described, particularly regarding the accuracy at which the root system and potential transpiration are simulated over time. Further studies dealing with long-term effects of rainfall regimes on soil water resources, could contribute to evaluating adaptive changes induced by drought periods in tropical *Eucalyptus* plantations.
plantations.

CO_2 production during fine root decomposition and root respiration

Simulations should include CO_2 gas emissions produced at each time step by fine roots (mortality and decomposition, root respiration, …). From these results, a comparison with the measurements of CO_2 performed throughout the soil profile need to be performed to assess the main gas transport processes and sources.

CO_2 production has been found to be correlated to root biomass throughout the soil profile (Chapter 6). Mechanistic biogeochemical models need to include the relationships between soil CO_2 production and soil water potential, soil temperature over time, but also fine root growth and root activity across soil layers. Indeed, incorporating the different level of processes of root respiration and microbial decomposition over depths should provide more accurate estimations and could reduce uncertainty in model simulations.

Prediction of CO_2 production in deep soil profiles under climate change

Simulations to explore the effects of future climate scenarios on CO_2 production and transfer in deep tropical soils could be performed including the current measurements as initial boundary conditions, and by comparing climatic series representing future climatic scenarios at the experimental field location. The predictions could be useful to assess qualitatively the consequences of climate change on CO_2 production in tropical eucalypt plantations growing on deep Ferralsols.

Conclusion

The modeling approach used in this thesis revealed promising efficiency in a model that couple root system architecture and functions, the Root Typ model, with reactive transport processes in soils, the Min3P model. Unfortunately, this thesis also revealed several issues that we could not overcome over the available time of 6 months at the end of the thesis. This chapter showed that additional experimental researches and model developments are required to assess the production of gas throughout the soil profiles and to infer the sensibility of gas production to

environmental factors in this *Eucalyptus* plantation. Therefore, a specific thesis in modeling is required to reinforce and further explore the possibilities of coupling Min3P and RootTyp to gain insight into the processes governing greenhouse gases transfer at a local scale.

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CHAPTER 8: GENERAL DISCUSSION AND PERSPECTIVES

The general objective of this study was to assess the effect of clear-cutting *Eucalyptus* stands managed in coppice on fine root production as well as CO_2, N_2O and CH_4 fluxes throughout the entire soil profile, down to the water table, with a focus at great depth. The study was carried out in *Eucalyptus* plantations in Brazil, which has already been proven to be an excellent model to understand more precisely aboveground and belowground ecophysiological and biogeochemical processes (Laclau *et al.*, 2010; Battie-Laclau *et al.*, 2016; Pradier *et al.*, 2017). Two contrasting water regimes were studied to gain insight into the effects of drought periods that are predicted to be more frequent over the next decades. The current, natural water regime (+ W) was compared to a reduced water regime (-W), simulating a reduction in precipitation of about one third. Fine roots and greenhouse gases were sampled down to the water table (here located at a depth of 17 m) through large and deep wells, over one year before the clear-cut and two years after harvesting, in coppice-managed plantations as well as down to a depth of 4 m in a non-harvested plot (NH) serving as a control.

This study aimed to test four hypotheses: 1/ *Eucalyptus* trees are able to adapt their fine root dynamics depending on their environment to better access the soil resources, 2/ a change in fine root dynamics can occur between the topsoil and deep soil horizons when trees are subjected to drought conditions, 3/ throughfall exclusion can significantly affect greenhouse gas production and consumption within the soil profile by modifying gas diffusivity, 4/ coppice management increases the efflux of GHG as a result of fine root mortality and decomposition of harvest residues. The consequences of tree harvesting on fine root dynamics and the production of greenhouse gases over the first year of the rotation in coppice-managed plantations ask specific questions never studied in very deep soil layers.

Through this work, we wanted to answer four scientific questions:

1. What are the dynamics of eucalypt fine roots, CO_2, N_2O and CH_4 concentrations throughout the entire soil profile down to a depth of 17 m?

2. Are fine root dynamics and fine root traits differently impacted by a change in water regime depending on soil depth?

3. What are the consequences of drought on greenhouse gas production in eucalypt plantations established in deep Ferralsols?

4. How clear-cutting affect root production and mortality but also greenhouse gas production in coppice-managed eucalypt plantations?

The work in this thesis consisted in collecting greenhouse gases down to a depth of 15.5 m, measuring greenhouse gas effluxes, collecting root samples down to the water table and monitoring root dynamics over 3 years (Chapters 5 and 6). This approach provided original results on fine root dynamics and greenhouse gas production in very deep soil layers in coppice-managed *Eucalyptus* plantation subjected to two contrasted rainfall regimes. A modeling approach was then used to gain insight into the processes governing CO_2 transfer at a local scale but revealed several issues that we could not overcome over the available time of 6 months at the end of the thesis (Chapter 7). Finally, this last chapter aims at discussing the results obtained throughout the thesis and is organized in two parts. In the first one, we discuss the contribution of deep roots in tree adaptation to drought conditions and the importance of considering the entire soil profile when evaluating stand-scale greenhouse gases emissions. In the second, we argue, according to an operational point of view, that coppice management might be an interesting option in tropical plantations to improve tree tolerance to drought and store carbon in deep soil layers.

VIII.1 Scientific discussion: contribution for global concepts

VIII.1.1 Effect of climate change on forest plantations: Let's look deeper!

Understanding the effects of climate changes, predicting its potential impacts and anticipating adaptation is mandatory for the sustainability of fiber and food production in a context of increasing human population. If greenhouse gas emissions maintain their current increase, climate change may increase the vulnerability of agricultural and forestry systems and reduce production in some parts of the world. Perennial plantations will be particularly affected by climate change, because a plot planted today must be able to cope with all the climatic conditions that will be encountered up to tree harvest, from a few years to several decades after planting. Predicting accurately the future climate is challenging. It requires complex models that consider many processes that can potentially greatly influence the predictions when the model is sensitive to some parameters. Yet, many processes are still not completely understood for deep rooted species, especially for the ones occurring at great soil depth. Deep rooting is common in tropical forests (Nepstad *et al.*, 1994; Canadell *et al.*, 1996; Jackson *et al.*, 2000) where severe drought events are predicted to occur over the next decades. In tropical forest

plantations, it is then crucial to focus the research efforts on the understanding of deep soil processes to assess the potential impacts of climate changes.

Current climate models predict a reduction in rainfall associated with an intensification of dry seasons in many parts of the word, and in Brazil particularly. Assessing how trees will adapt to climate changes is a major challenge. Therefore, field experimentations simulating future climates are crucial to parameterize biogeochemical global models making it possible to predict how ecosystems will respond to climate changes.

Anthropogenic climate changes are caused mainly by greenhouse gas (GHG) emissions. GHGs refer to all gases of natural or anthropogenic origin that contribute to the greenhouse effect. Potential efficiency of greenhouse gases regarding global warming is highly variable. By definition, the overall global warming potential of CO_2 is equal to 1 and CH_4 and N_2O are 23 and 298 times more efficient to warm the earth over a 100-year period as compared to CO2, respectively. In the early 1990s, through the multiplication of scientific evidence and international conferences on climate (including the conference of Kyoto in 1997), the international community has become aware of the potential importance for climate changes of increasing concentrations of anthropogenic GHGs in the atmosphere (mainly CO_2, N_2O and CH_4) and the need to reduce them. To reduce the concentrations of GHGs in the atmosphere, two types of actions can be considered:

1. Decrease sources, in other words, reduce the level of GHG emissions.
2. Increase sinks, in other words, favor the transfer and storage of GHGs in other compartments where they would be stabilized, for example, in the form of carbonate sediments, woody plant biomass and / or stabilized organic matter.

Our study puts forward the need to consider deep soil layers to evaluate both actions. Indeed, it is mandatory to study deep soil layers in order to quantify accurately C source/sink fluxes as part of the global carbon budget. Quantifying C fluxes in deep soil layers is needed to improve the biogeochemical models predicting the effect of drought periods on greenhouse gas effluxes, and to identify more sustainable silvicultural practices for tropical planted forests in a context of climate changes.

VIII.1.2 Looking deeper to quantify sinks accurately

Our study in a 2-ha split-plot experimental design manipulating water availability contributed to evaluating the impact of rainfall reduction on the productivity and adaptability of *Eucalyptus* plantation in a context of global change. Our results clearly show that *Eucalyptus* trees facing drought invest more in belowground biomass to increase the exploration of fine roots in deep soil layers (Figure VIII. 1). This adaptive strategy to drought stress of perennial plant species, investing assimilates to develop the deep root system, increases the availability of water stored in very deep soil layers to maintain tree growth during drought periods (Christina *et al.*, 2017). Furthermore, the increased surface of root in contact with soil through an adjustment of fine root morphology (SRA, SRL, diameter, Figure VIII. 1) reflects a significant expression of root plasticity in response to limited soil resources (Hodge, 2004; Fort *et al.*, 2015; Roumet *et al.*, 2016).

A higher investment in deep roots in response to a rainfall reduction might also influence other key processes such as tree functional status (i.e. growth and senescence), C rhizodeposition and rhizosphere biogeochemical properties. For instance, we found a non-negligible production of greenhouse gases down to the water table, probably resulting from the respiration of fine roots and their decomposition at depths of more than 10 m. On the other hand, deep roots might also contribute to sequestrating large amounts of C in soils (Pierret *et al.*, 2016). We showed that fine root biomass increased in deep soil layers when the plantation was subjected to water deficit (Figure VIII. 1). As long-term C sequestration is more likely to occur in deep soil horizons than in the topsoil (Gill and Burke, 2002), the increased C allocation to fine roots in deep soil layers could have significant role for mitigating global changes.

Consequently, because changing precipitations can greatly modify C allocation between soil layers, C sequestration must be studied not only the top soil, but also throughout the whole soil profile explored by roots.

VIII.1.3 Looking deeper to quantify sources accurately

Several studies reported that total below-ground carbon allocations account for 20-30% of gross primary production in *Eucalyptus* plantations (Ryan *et al.*, 2010; Epron *et al.*, 2012; Nouvellon *et al.*, 2012). The results of our study emphasize that *Eucalyptus* roots do not only represent a non-negligible part of the biomass of the trees, but also that a large proportion is allocated at great depth, especially under drought conditions (Figure VIII. 1). This is particularly of interest considering that plants play a major role in sequestrating CO_2 from the atmosphere into the soil.

In order to assess the carbon fluxes from the soil to the atmosphere, we conducted measurements of CO_2 fluxes along the soil profile and at the soil surface.

CO_2 productions estimated with the diffusivity model were strongly correlated to the distribution of fine root biomass along the soil profile, down to a depth of 15.5 m. The CO_2 production shifted to deep soil layer in response to throughfall exclusion, which could be due to a shift of root growth to deep layers likely to enhance tree water supply (Sotta *et al.*, 2007; Schwendenmann *et al.*, 2010). The higher CO_2 production at some depth in -W plots than in +W plots could result from a higher labile carbon production when fine roots are active, because there is more exudation, respiration and necromass decomposed by microbes. When decomposed, a part of the dead root tissues is incorporated into the soil organic matter (Strand *et al.*, 2008). Microorganisms can be reactivated in response to the input of fresh organic matter. Specialized microorganisms develop rapidly and break down fresh organic matter. Their metabolites can activate a second class of specialized microorganisms, in dormancy, which can degrade the organic matter initially present and stored in the soil: this is the "priming effect" (Fontaine *et al.*, 2003). We observed a decrease in greenhouse gas production in the upper layers and an increase in the deep layers in response to throughfall exclusion. Changes in deep soil conditions in response to water deficit might affect considerably the functioning and composition of microbial communities and therefore the production of CH_4, CO_2 and N_2O. A study down to a depth of 4 m at the same site showed that total organic C and N concentrations in the rhizosphere increased with depth and in response to throughfall exclusion (Pradier, 2016). This increase can be explained by two major processes: (i) the increase in the quantity of organic compounds released by deep roots, and/or (ii) an identical amount of organic compounds released but a difference in their residence time within the rhizosphere (Pradier, 2016). Those results are consistent with the significant production of greenhouse gases observed in this study down to a depth of 15.5 m (Figure VIII. 2). It is therefore essential to clarify the functional role of deep roots and associated microbial communities to estimate adequately the overall C sequestration and greenhouse gas emissions in tropical ecosystems.

Furthermore, the production of greenhouse gases shifted over depth under dry conditions in this study, showing that it is important to simulate the changes along the entire soil profile in biochemical models to estimate CO_2 emissions under future conditions. Therefore, only considering greenhouse gas production in the top soil layers will not be adequate to evaluate the impact of climate changes in tropical forest ecosystems over long time periods.

VIII.2 Operational discussion: consequences for the management of tropical planted forests

Eucalyptus plantations are not natural forests and are devoted to the provision of wood products, as viticulture is devoted to wine production or coffee plantations to coffee production. The sustainability of *Eucalyptus* plantations requires the control of criteria and indicators of sustainable ecological and social management practices, but also economic and institutional aspects. *Eucalyptus* plantations cover large areas worldwide, with more than 20 million hectares. The large production of wood in fast-growing plantations helps to reduce the pressure on native forest to supply wood and especially the high emissions of greenhouse gases caused by deforestation. However, much remains to be done regarding environmental impacts, sustainable management and adaptation to global change in order to reconcile production issues and preservation of natural resources. In this perspective, our study contributes to demonstrating that coppice management of *Eucalyptus* plantations could be an interesting trade-off between economic, societal and environmental requirements.

VIII.2.1 Coppice-managed forest plantations: an option to limit GHG emissions

Regeneration of forest plantation by sprouting is a viable alternative for many production systems. More and more forest companies opt for coppice-managed *Eucalyptus* plantations instead of systematic replanting for cost and profitability reasons (Gonçalves *et al.*, 2014). About one third of commercial *Eucalyptus* plantations in Brazil have been managed in coppice management since 2010 (IBA, 2016). However, the consequences of this type of management on the functioning of deep roots have never been investigated in tropical planted forests.

Such a research effort is a prerequisite to orient the management of tropical planted forests toward a limitation of greenhouse gas emissions in the atmosphere. Our study shows that managing the eucalypt plantations in coppice after cutting the previous stand did not led to an increase in CO_2, CH_4 and N_2O emissions (Figure VIII. 2). Fine root mortality was very low the first year after the clear-cut when the plantation was coppice-managed (Figure VIII. 1). The management in coppice after the clear-cut did not increase the production of dead roots and the subsequent release of CO_2 resulting to fine root decomposition. Furthermore, coppiced-managed plantations are less prone to erosion compared to replanted stands because the soil is exposed for a shorter period (Berhongaray *et al.*, 2017). Hence, coppice management might be

an interesting option in tropical *Eucalyptus* plantations, to help mitigate the increase in CO_2, CH_4 and N_2O in the atmosphere and to limit soil erosion compared to systematic replanting.

VIII.2.2 Coppice-managed forest plantations: an option for areas of climatic constraints

Our study also showed that coppice management for *Eucalyptus* plantation might be a promising option to face water scarcity. Indeed, we found an intensive root growth in very deep soil layers (depth > 14m) less than one year after the clear-cut, with only few roots lost by mortality. These findings are particularly impressive considering that *E. grandis* roots typically reach only a depth of 7 m at one year after planting in systematic replanting (Christina *et al.*, 2011; Laclau *et al.*, 2013). Root production and maintenance represent a significant cost to a plant's overall economy. The establishment of a deep root system requires the allocation belowground of large amounts of carbon, which is at the expense of the production of aboveground biomass. By maintaining the root system, coppice-managed *Eucalyptus* plantations preserve belowground biomass for the next generation. Our results suggest that the carbon cost to maintain the root system down to the water table could be lower than shedding roots and producing new roots when the plantation is coppice-managed. Six-year-old *Eucalyptus grandis* trees in our experiment have enough reserves within the root system, with or without throughfall exclusion, and the appropriate edaphic surrounding conditions to maintain the fine root biomass after clear-cutting, which probably contributed to promoting the early shoot growth (Drake *et al.*, 2013; Brunner *et al.*, 2015).

The maintenance of a deep root system represents a double "safety net" for nutrients and for water uptake that are taken up throughout the entire soil profile before being lost for tree growth by deep drainage (Mareschal *et al.*, 2013). In tropical *Eucalyptus* plantations growing on highly weathered soils in regions with rainfall events of high intensity, deep roots can provide access to large amounts of water stored in the subsoil and can play a crucial role in nutrient capture, which is essential to avoid large losses of nutrients leached from the upper horizons (Laclau *et al.*, 2010). Therefore, the pre-established root system in coppice-managed plantations can act as a safety net by catching up large amounts of nutrient released after tree harvest and probably contribute to reducing the risk of tree mortality during prolonged dry periods.

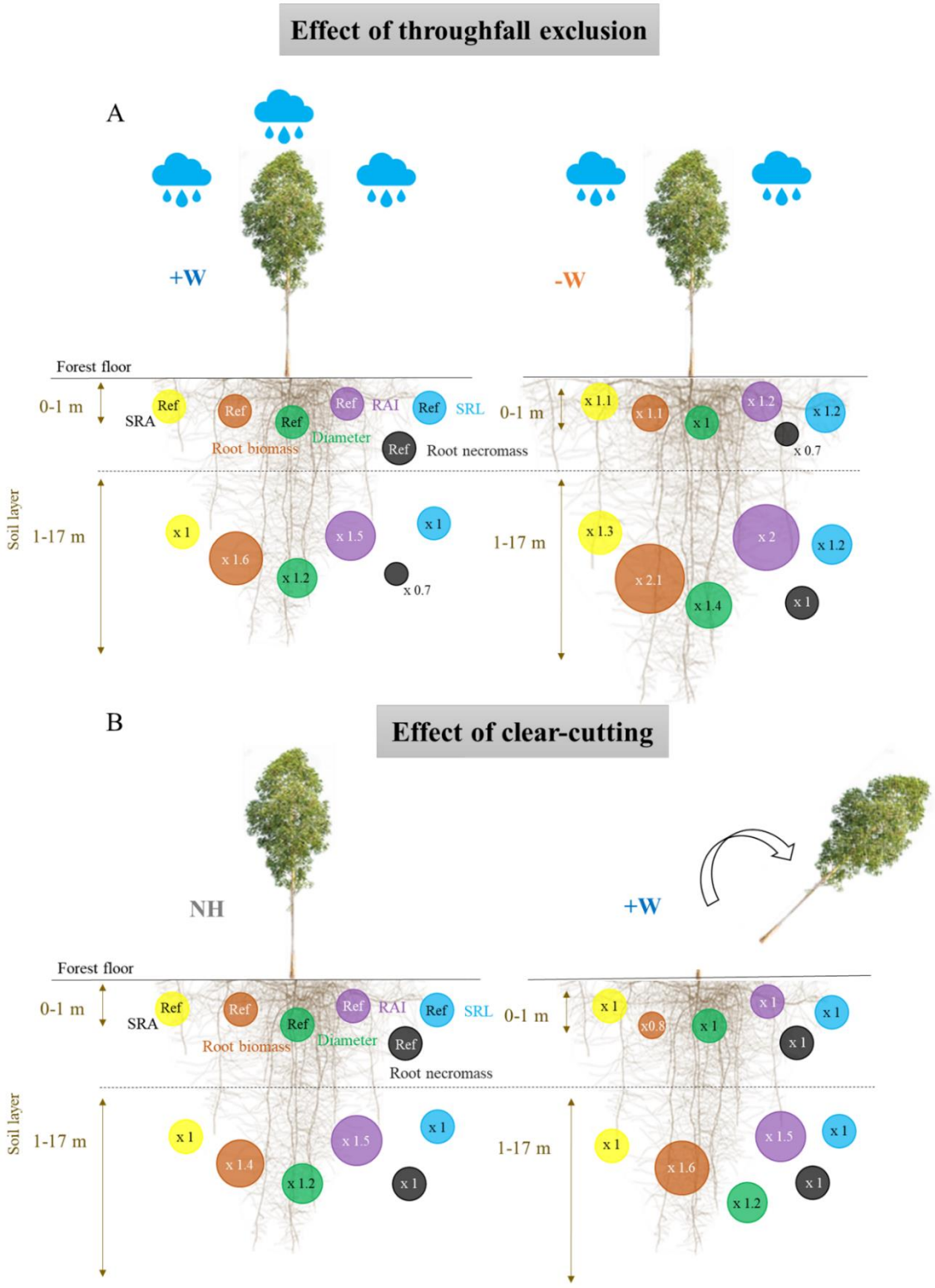

Figure VIII. 1: Conceptual representation of the influence of A) throughfall exclusion and B) clear-cutting on mean specific root area (yellow), total root biomass (orange), mean diameter (green), mean root area index (purple), total root necromass (black) and mean specific root length (blue) across deep soil profiles. The size of the circles is not at scale, the idea is only to visualize trends between soil layers 0-1 m, 2-6 m and 10-16 m, and between treatments +W, -W and NH. Ref in A) and B) are the reference values.

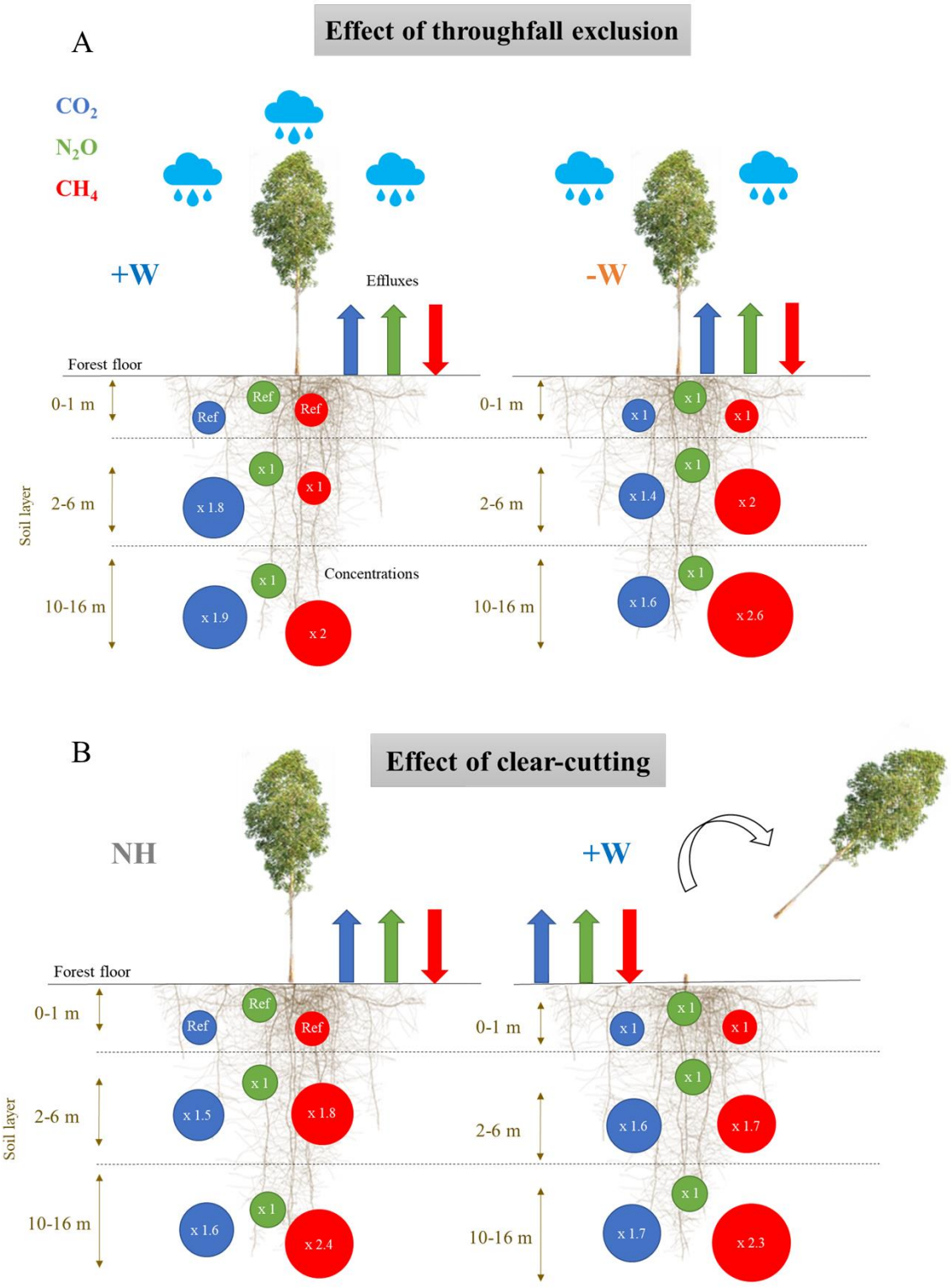

Figure VIII. 2: Conceptual representation of the influence of A) throughfall exclusion and B) clear-cutting on mean CO_2 (blue), N_2O (green) and CH_4 (red) concentrations across deep soil profiles. The size of the circles is not at scale, the idea is only to visualize trends between soil layers 0-1 m, 2-6 m and 10-16 m, and between treatments +W, -W and NH. Ref in A) and B) are the reference values. The arrows at soil surface indicate whether the net gas efflux from soil to atmosphere is positive or negative.

VIII.3 Contributions of the study and perspectives

The outputs of this work are multiple (Figure VIII. 1, Figure VIII. 2) To our knowledge, it is the first time that fine root dynamics and greenhouse gas concentrations and productions are studied down to the water table (here at a depth of 17 m) in *Eucalyptus* plantations. The effects of water deficit in interaction with the consequences of clear-cutting in coppiced-managed *Eucalyptus* plantations have never been studied.

However, further studies dealing with the anatomical, architectural and functional characteristics of fine roots across very deep soil profiles should be carried out. These studies would help gain an insight into the potential impact of management practices on C, water and nutrient cycles in tropical regions. A better knowledge of microbial communities in terms of biomass and diversity, but especially in terms of metabolic capacity is also needed. Improving our understanding of the factors controlling microbial activity, nutrient and C availability and their interactions in deep soil layers is a major scientific lock to model the global biogeochemical cycles (Fierer *et al.*, 2003; Jones *et al.*, 2018; Sosa-Hernandez *et al.*, 2018).

From a methodological point of view, the field measurements in our study showed the great variability of GHG effluxes and the importance of monitoring the determinants of these emissions. Unlike micro-meteorological techniques, the closed-chamber method is simple, relatively inexpensive and can be installed on any type of field (forest, cultivated field, grassland ...). Problems related to the use of closed-chambers (leaks, overpressure ...) can be avoided or minimized by an adapted methodology. The main limitation of this technique is the small area covered by the measurement, which may not encompass large-scale processes. A large number of samples in each treatment were set to catch the potential intra-plot variability, but those sampling efforts required a significant labor load and the measurements remained punctual in time and space. The use of automatic chambers allows to better capture the temporal fluctuations of greenhouse gas emissions (Makita *et al.*, 2018). This is particularly an advantage in places where environmental factors, contributing to the regulation of greenhouse gas emissions (such as soil temperature), lead to large differences in daytime / nighttime GHG effluxes.

In situ measurements are needed to explore the dynamics of greenhouse gas concentrations throughout soil profiles. However, manual *in situ* measurements require a lot of time and resources and are therefore often limited in time and space. Modeling makes it possible to interpolate in space and time the data acquired punctually. These interpolations are particularly

interesting for the greenhouse gas effluxes that are characterized by strong spatial and temporal variations, as observed in this study. The sensibility of model outputs to specific processes can also be assessed by analyzing the differences between experimental and simulated data. Moreover, models can be used to assess the consequences of contrasting scenarios on greenhouse gas production. Nevertheless, the mechanistic modeling of GHG effluxes still presents many uncertainties because the main factors used in the models (mineral N dynamics, water contents, organic C contents, soil temperatures, soil pH, …) and their interactions are not fully understood, in particular throughout deep soil profiles. Understanding how precipitation changes, and especially extended drought periods, influence greenhouse gas production in deep soils is crucial to improve the prediction of terrestrial biosphere models.

While many studies continue to consider only the upper 30 centimeters of soil, this work highlights the urgent need to study deep soil horizons in a context of climate change, not only for the evaluation of the changes in soil carbon sequestration, but also to quantify accurately the production of greenhouse gases. Comprehensive studies dealing with the microbial ecology of deep soil horizons in response to environmental changes and management practices are crucial to predict the behavior of huge soil volumes over the next decades.

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IX. SUMMARY OF THE SCIENTIFIC CONTRIBUTIONS OF THE THESIS

Scientific publications

An article published as the first author during the thesis, full version presented below:

Germon, A., Guerrini, I.A., Bordron, B., Bouillet, J.-P., Nouvellon, Y., de Moraes Gonçalves, J.L., Jourdan, C., Paula, R.R., Laclau, J.-P., 2018. Consequences of mixing *Acacia mangium* and *Eucalyptus grandis* trees on soil exploration by fine-roots down to a depth of 17 m. Plant and Soil 424, 203-220.

An article published as a co-author during this thesis:

Lambais, G.R., Jourdan, C., de Cássia Piccolo, M., Germon, A., Pinheiro, R.C., Nouvellon, Y., Stape, J.L., Campoe, O.C., Robin, A., Bouillet, J.-P., 2017. Contrasting phenology of *Eucalyptus grandis* fine roots in upper and very deep soil layers in Brazil. Plant and Soil 421, 301-318.

Articles submitted as co-author during this thesis:

Robin A., Pradier C., Sanguin H., Mahé F., Lambais G.R., Pereira A.P.A, Germon A., Santana M.C., Tisseyre P., Pablo A-L., Heuillard P., Sauvadet M., Bouillet J-P., Andreote F.D., Plassard C., Gonçalves J.L.M, Jurandy E., Cardoso B.N., Laclau J-P., Hinsinger P., Jourdan C. How deep can ectomycorrhizas go? A case study on *Pisolithus* down to 4 meters in a Brazilian eucalypt plantation. *Submitted to Mycorrhiza* 2019

Bordron, B., Germon, A., Laclau, J-P., Rosada, I., Robin, A., Jourdan, C., Paula, RR., Pinheiro, RC., Guillemot, J., Gonçalves, JLM., Bouillet, J-P. *Eucalyptus* fines roots development in mixed species plantation shows higher Acacia N facilitation under the stress gradient hypothesis. In prep.

Communications in international congress

1. Lightening talk at EcoSummit 2016 congress in Montpellier from 29 August to 1 September 2018: “Consequences of mixing *Acacia mangium* and *Eucalyptus grandis* trees on soil exploration by fine roots down to a depth of 17 m in a tropical planted forest”
2. Poster presentation the IUFRO 2017 congress in Germany from 18 to 22 September 2017: “Consequence of clear-cutting and drought on deep soil CO_2 and N_2O concentrations down to a depth of 16 m in Brazilian eucalypt plantations”
3. Oral presentation at the ISRR 2018 congress in Israel from 8 to 12 July 2018: “Consequences of clear-cutting and drought on fine root production and mortality down to 17 m in Brazilian eucalypt plantations”
4. Oral presentation at *Eucalyptus* 2018 congress in Montpellier from 17 to 21 September 2018: “Dynamics of CO_2, CH_4 and N_2O concentrations throughout deep soil profiles in Eucalypt plantations subjected to contrasted rainfall regimes: consequences on soil effluxes”

Supplementary Article

In addition to the studies presented in this thesis, during my thesis, I had the opportunity to study the consequences of mixing *Acacia mangium* and *Eucalyptus grandis* trees on soil exploration by roots down to the water table at 17 m depth in a tropical planted forest.

Eucalyptus plantation mixed with nitrogen fixing trees can be considered as a form of ecological intensification through the increase of forest plantations production and nutrient availability in the soil (Bouillet et al. 2012, Forrester et al., 2005; Kelty, 2006). Multi-purpose plantations can provide key ecosystem services (Paquette & Messier, 2010), in some situations, increase the total biomass production (Forrester, 2014; Richards et al., 2010) and positive relationships between tree diversity and soil fauna, microbial diversity, and soil carbon (C) sequestration are well documented (Blaser et al., 2014; Forrester, 2014; Richards et al., 2010). But the consequences of mixing trees on root growth and fine-root morphology in very deep soil layers is poorly know.

In this study, root sampling was realized down to 17 m in *A. mangium* and *E. grandis* monospecific stands and a mixture with 50% of each species using the method described in chapter 5. In this study, I did not contribute to the acquisition of the data, but I contributed to the data analysis as well as the statistical analyses and the writing of the paper.

This article is intitled “Consequences of mixing *Acacia mangium* and *Eucalyptus grandis* trees on soil exploration by fine-roots down to a depth of 17 m”. Co-authors associated with this project are Iraê Amaral Guerrini, Bruno Bordron, Jean-Pierre Bouillet, Yann Nouvellon, José Leonardo de Moraes Gonçalves, Christophe Jourdan, Ranieri Ribeiro Paula and Jean-Paul Laclau.

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Consequences of mixing *Acacia mangium* and *Eucalyptus grandis* trees on soil exploration by fine-roots down to a depth of 17 m

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Key words: plantation, forest, deep root, fine-root density, root traits, diversity, over-yielding, Brazil

Abstract:

Background and Aims Fine-root functioning is a major driver of plant growth and strongly influences the global carbon cycle. While fine-root over-yielding has been shown in the upper soil layers of mixed-species forests relative to monospecific stands, the consequences of tree diversity on fine-root growth in very deep soil layers is still unknown. Our study aimed to assess the consequences of mixing *Acacia mangium* and *Eucalyptus grandis* trees on soil exploration by roots down to the water table at 17 m depth in a tropical planted forest.

Method Fine roots (diameter < 2 mm) were sampled in a randomized block design with three treatments: monospecific stands of *Acacia mangium* (100A), *Eucalyptus grandis* (100E), and mixed stands with 50% of each species (50A50E). Root ingrowth bags were installed at 4 depths (from 0.1 m to 6 m) in the three treatments within three different blocks, to study the fine-root production over 2 periods of 3 months.

Results Down to 17 m depth, total fine-root biomass was 1127 g m^{-2} in 50A50E, 780 g m^{-2} in 100A and 714 g m^{-2} in 100E. Specific root length and specific root area were 110-150% higher in 50A50E than in 100A for *Acacia mangium* trees and 34% higher in 50A50E than in 100E for *Eucalyptus grandis* trees. Ingrowth bags showed that the capacity of fine roots to explore soil patches did not decrease down to a depth of 6 m for the two species.

Conclusions Belowground interactions between *Acacia mangium* and *Eucalyptus grandis* trees greatly increased the exploration of very deep soil layers by fine roots, which is likely to enhance the uptake of soil resources. Mixing tree species might therefore increase the resilience of tropical planted forests through a better exploration of deep soils.

IX.1 Introduction

Plantations of fast-growing trees are expanding rapidly to meet the increasing demand for wood products (Keenan et al., 2015). The area covered by planted forests increased from about 168 million ha in 1990 to 278 million ha in 2015, and a large share of the new forest plantations are at sites with in highly weathered tropical soils (Keenan et al., 2015). Fast-growing *Eucalyptus* plantations cover about 20 million hectares throughout the world (Booth, 2013) with about 5.6 million hectares in Brazil (IBA 2016). *Eucalyptus* plantations provide raw material for pulp and paper, charcoal and firewood in Brazil (Battie-Laclau et al., 2014; Gonçalves et al., 2013). While most of these eucalypt plantations are monospecific, mixed-species stands including nitrogen (N) fixing trees can help to balance the N budget, improve the N status of the eucalypts (Forrester et al., 2006; Paula et al., 2015) and, in some situations, increase the total biomass production (Forrester, 2014; Richards et al., 2010). Multi-purpose plantations can provide key ecosystem services (Paquette & Messier, 2010), and positive relationships between tree diversity and soil fauna, microbial diversity, and soil carbon (C) sequestration are well documented (Blaser et al., 2014; Forrester, 2014; Richards et al., 2010). *Acacia mangium* Wild is another fast-growing tree species widely planted in South-East Asia for pulpwood. Introduction of *Acacia mangium* trees into commercial eucalypt plantations has been tested recently in Brazil and Congo (Bouillet et al., 2013; Santos et al., 2016; Voigtlaender et al., 2012). Those studies showed that mixed *A. mangium* and *Eucalyptus* stands had higher available mineral N in the soil than monospecific eucalypt stands (Tchichelle et al., 2017; Voigtlaender et al., 2012) and that, in some situations, the total biomass production was higher in mixed stands than monospecific eucalypt stands at the same stocking density (Bouillet et al., 2013; Epron et al., 2013; Santos et al., 2016). While the benefits consequences of a stratified canopy in mixed-species plantations are well documented (Bauhus et al., 2004; Binkley et al., 2013; Le Maire et al., 2013; Williams et al., 2017), soil partitioning between the roots of different tree species in a mixed stand has not been studied to the same extent.

Roots play a key role in forest ecosystems with their mechanical functions of tree anchorage and their physiological function of capturing and transporting the amounts of water and nutrients needed for plant growth (McCormack & Guo, 2014; Pregitzer et al., 2002). Niche complementary among the fine roots of different species is often cited as one of the major processes that can contribute to increasing biomass production in multi-species stands compared to single-species forests (Lehmann & Schroth, 2003). Studies dealing with fine-root density in mixed-species forests are scarce and mainly limited to the upper soil layers. Deep

roots, commonly defined as roots growing below 1 m, can provide access to water and nutrient pools that shallow roots cannot reach (Kell, 2012). Even though the density of roots is generally low below 1 m depth compared to the topsoil, they are important in reducing nutrient losses by deep drainage (Laclau et al., 2010) and improving trees' drought tolerance to drought (Christina et al., 2017a; McDowell et al., 2008). While the effects of environmental changes on the phenology of aboveground plant components are well documented, belowground processes remain poorly understood (Radville et al., 2016b). The lack of information on the influence of interspecific interactions on root exploration at great depth limits our ability to identify the most appropriate sites for multi-species plantations in tropical regions. Fine-root biomass in the 0-2 m soil layers was found to be 30% higher in a mixed-species plantation of *E. grandis* and *A. mangium* trees than in monospecific stands at age 5 years in Brazil (Laclau et al., 2013c). Interaction between the two-species led to a segregation of the root systems. In mixed stands, *A. mangium* fine roots were partially excluded from the topsoil in mixture and over-explored the soil layers between the depths of 1 and 2 m relative to monospecific *A. mangium* stands (da Silva et al., 2009; Laclau et al., 2013c). However, as far as we are aware, the effects of the inter-specific interaction on fine-root densities at depths > 2 m have never been studied. Changes in fine-root traits might be a major adaptation of *A. mangium* and *E. grandis* trees to cope with inter-specific interactions in mixed stands. Strong modifications of fine-root morphology have been shown in response to competition through an increase in specific root length (SRL, fine-root length divided by fine-root dry mass) and specific root area (SRA, fine-root area divided by fine-root dry mass) to reduce the cost/benefit ratio of resource capture.

Root growth is controlled by endogenous constraints on carbon availability and environmental factors (Freschet et al., 2017; McCormack et al., 2015). Fine-root production is dependent on soil water content (Canham et al., 2015) and the high plasticity of fine roots enables them to explore resource-rich soil patches has been demonstrated (Hodge, 2004). The capacity of *A. mangium* and *E. grandis* roots to explore soil patches might therefore vary depending on the season and the depth in the soil. Although ingrowth bags have been commonly used to estimate fine-root production in forest ecosystems (Brunner et al., 2013), the root-free soil in the ingrowth bags can be richer in nutrients and water than the surrounding soil (without water and nutrient uptake in the first weeks after installation), which can lead to overestimates of the fine-root production and length in these soil patches relative to the surrounding soil (Bauhus & Messier, 1999; Jourdan et al., 2008).

Our study aimed to assess the consequences of mixing *Acacia mangium* and *Eucalyptus grandis* trees on fine-root growth down very deep profiles in highly weathered tropical soils. We hypothesized that the interaction between *Acacia mangium* and *Eucalyptus grandis* trees in mixed stands modified root growth relative to monospecific stands, resulting in i) fine-root over-yielding in all the soil layers down to the water table at a depth of 17 m, ii) modification of common fine-root traits (diameter, SRL, SRA) for the two species making it possible to increase the area of soil resource capture per gram of C invested in fine roots, and iii) a higher capacity of the tree roots to explore soil patches in the topsoil than in very deep soil layers reflecting the decrease in fine-root density with depth.

IX.2 Materials and methods

IX.2.1 Study site

The study was carried out at the Itatinga experimental station of University of São Paulo, Brazil (23°02' S., 48°38' W., 860 m a.s.l.). This region has a humid subtropical climate (Cfa according to the Köppen classification) with an average annual rainfall of 1390 mm (mean from 1990 to 2010), a mean relative humidity of 77% and a mean annual temperature of 19°C with a cold and dry season from June to September. The soils are Ferralsols (FAO classification) developed on Cretaceous sandstone with a water table at a depth of 17 m (Pradier et al., 2017). Sand content was around 85% in the topsoil and 75-80% between 1 m and 15 m depth (Maquere, 2008). Soil pH_{H2O} was approximately 5.5 in the upper 3 m, cation exchange capacity decreased from 18 $mmol_c\ kg^{-1}$ in the 0-5 cm soil layer to 2 $mmol_c\ kg^{-1}$ in the 2-3 m layer, and exchangeable cation contents dropped below a depth of 5 cm. Full details were given in a previous study (Laclau et al., 2013c).

IX.2.2 Experimental Layout

The trial was a complete randomized block design with 7 treatments and 4 blocks set up in May 2003 in a site which had been a *Eucalyptus grandis* plantation from 1998 to 2002 and previously a *Eucalyptus saligna* Sm. coppice from 1940 to 1998. The experimental layout was described in detail by Laclau et al. (2008) and Le Maire et al. (2013). There were plots with *A. mangium* monoculture, *E. grandis* monoculture and mixed plantations of *A. mangium* and *E. grandis* within each block. Each plot was 30 m x 30 m with two buffer rows. The seedlings were planted at a density of 1111 trees ha^{-1} (3 m x 3 m spacing). The trees were harvested at age 6 years (in

May 2009) and only the harvested boles were removed from the plots. Harvest residues were spread uniformly over each plot and *A. mangium* and *E. grandis* seedlings were re-planted in November 2009 in the same planting rows of the same plots, at 50 cm from the stumps of the previous rotation.

Our study was carried out 4 years after replanting in 3 treatments within 3 blocks: *A. mangium* and *E. grandis* monospecific stands (100A and 100E, respectively) and mixed stands with 50% of each species (50A50E). In the mixed stand, the two species were planted alternately in the row and offset adjacent rows (555 trees ha^{-1} per species). The *Acacia mangium* seedlings were inoculated with rhizobia strains selected by EMBRAPA (Agrobiology, Rio de Janeiro) for their N_2 fixation capacities. The same amounts of P, K, Ca, Mg, and micronutrients were applied the first 18 months after planting in all the plots (no N fertilization). Destructive sampling at age 3.3 years showed that mixing the two species did not lead to higher aboveground biomass in 50A50E than the average of the two monocultures. Tree height was about 8 m higher for *E. grandis* than for *A. mangium* (Table IX. 1).

Table IX. 1: Main characteristics of the stands at 39 months (mean and standard deviation between blocks, n=3). Different uppercase letters indicate significant differences between treatments, and different lowercase letters indicate significant differences between the monospecific stands and the mixed stands for each species ($p < 0.05$). The methods used to estimate the aboveground biomass are described in Nouvellon et al. (2012b).

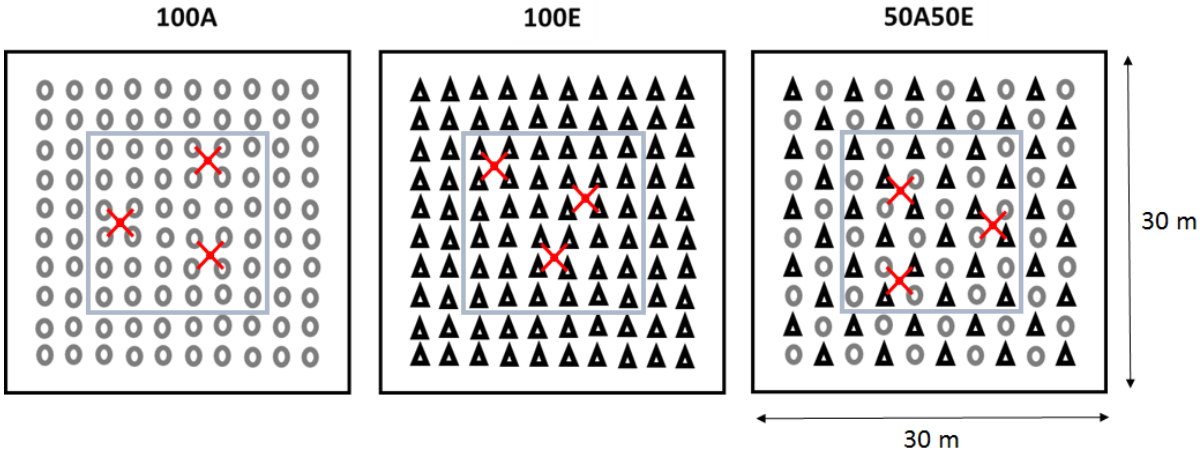
	100A	**100E**	**50A:50E**		
	A. mangium	*E. grandis*	*A. mangium*	*E. grandis*	Total
Stocking density (trees ha^{-1})	988 ± 18	1111 ± 0	535 ± 21	556 ± 0	1091 ± 15
Stand basal area (m^2 ha^{-1})	15.3 ± 0.4 B a	16.6 ± 0.4 A a	7.1 ± 0.8 b	9.3 ± 0.6 b	16.4 ± 0.2 A
Tree height (m)	9.3 ± 0.2 C a	17.8 ± 0.2 A a	9.5 ± 0.3 a	16.8 ± 0.1 b	13.1 ± 0.2 B
Biomass (kg m^{-2}):					
Leaves	0.66 ± 0.02 A a	0.58 ± 0.02 B a	0.22 ± 0.03 b	0.46 ± 0.03 b	0.67 ± 0.01 A
Branches	0.83 ± 0.02 A a	0.81 ± 0.02 A a	0.31 ± 0.04 b	0.57 ± 0.07 b	0.88 ± 0.03 A
Bark	0.53 ± 0.01 C a	0.67 ± 0.02 A a	0.18 ± 0.02 b	0.43 ± 0.02 b	0.62 ± 0.00 B
Wood	1.99 ± 0.05 C a	4.96 ± 0.13 A a	0.80 ± 0.09 b	2.29 ± 0.12 b	3.09 ± 0.03 B
Aboveground biomass	4.01 ± 0.09 C a	7.03 ± 0.19 A a	1.51 ± 0.17 b	3.75 ± 0.24 b	5.26 ± 0.07 B

IX.2.3 Root sampling

Fine roots (diameter < 2 mm) were sampled down to a depth of 17 m in the three treatments (100A, 100E and 50A50E; Figure IX. 1) within the three blocks. Three replicates of soil samples were collected at mid distance between 4 adjacent trees (Figure IX. 1) in each plot down to a depth of 6 m and one replicate per plot between the depths of 6 m and 17 m (total of 9 samples per treatment in each soil layer down to 6 m depth and 3 samples between the depths of 6 m and 17 m). At each sampling position, soil layers 0-0.5, 0.5-1.0, 1.0-1.5, 1.5-2.0, 2.0-3.0, 3.0-4.0, 4.0-5.0, 5.0-6.0 m were collected by digging a square hole of approximately 15 cm x 15 cm area at the soil surface (an operator used a tool designed to dig very deep holes, called 'cavadeira' in Brazil). Only the soil mass sampled was measured accurately, the volume of the soil samples extracted using the 'cavadeira' tool could not be measured and was estimated by multiplying the soil dry mass of the samples by the bulk density in each layer (see below). About 15 kg and 30 kg of soil were collected at each sampling position for layers 50 cm and 100 cm thick, respectively. Fresh soil mass was measured in the field (±10 g) and all the fine roots easily visible were collected. The soil was then homogenized and a sub-sample of approximately 0.5 kg in the 0-50 cm soil layer, 1 kg in soil layers 0.5-1.0, 1.0-1.5, 1.5-2.0, 2.0-3.0, 3.0-4.0 m, and 2 kg in soil layers 4.0-5.0 and 5.0-6.0 m was taken to separate short lengths of root and small diameter fine roots in the laboratory. Each sub-sample was weighed in the laboratory and the soil water content was measured (by drying 5 g of soil at 105°C for 72 hours).

Below a depth of 6 m, soil cores were taken every 1 m depth using a cylindrical auger with an inner diameter of 9 cm and a length of 30 cm. We used the same methodology as Christina et al. (2011a) to avoid contamination of the soil samples collected at depth by roots from the upper layers. Only soil blocks from the inner part of the auger were collected and all fragmented soil pieces likely to come from upper soil layers were systematically discarded. All the soil collected from each layer was put in plastic bag, identified and stored at 4°C until processing (within 2 months after sampling).

Figure IX. 1: Layout of the three treatments studied: *Acacia mangium* and *Eucalyptus grandis* monospecific stands (100A and 100E, respectively) and a mixed stand with 50% of each species (50A50E). *A. mangium* trees are represented by grey circles and *E. grandis* trees by black triangles. Each inner plot (excluding two buffer rows) is delimited by a grey square. Three replicates samples (position indicated by a red cross) of soil cores were collected at mid distance between 4 adjacent trees in each plot down to a depth of 6 m and one sample per plot between the depths of 6 m and 17 m. Only one block is presented. A complete scheme of the trial is presented on Fig. 1 in Le Maire et al. (2013).



All the fine roots in the samples were washed free of soil with tap water using sieves (with mesh sizes from 0.50 to 1.19 mm) and all the living roots with a length >1 cm were separated carefully by hand. Dead roots separated by sight, touch and flotation, if required were discarded. Living roots were selected by various criteria such as living stele, bright color and elasticity. The color of the roots and the ramification pattern were good indications of the species to which they belonged. Reference roots sampled in monospecific stands were used to facilitate the identification of each species for roots collected in the 50A50E treatment. E. grandis fine roots were more branched and were darker than A. mangium fine roots. A sub-sample (10% of the weight of each soil sample) was used to estimate the mass of extremely fine roots (pieces of roots less than 1 cm in length). Extremely fine roots were separated carefully by hand in a white bucket filled with tap water. The mass of short root fragments (less than 1 cm in length) measured in 10% of the soil sample mass was multiplied by 10 to estimate the mass of those root fragments in the whole soil sample. All living fine roots with a length >1 cm separated from each soil sample were scanned (400 dpi resolution). Nodules were scanned with the fine roots when they were present. Root lengths and areas were estimated in each sample using WinRHIZO Version Pro V.2009c software (Regent Instruments, QC, Canada). Fine roots were dried for 72 h at 65 °C and weighed (±0.1 mg) to estimate specific root length (SRL, length of scanned roots divided by their dry mass mass, expressed in m g^{-1}) and specific root area (SRA, surface area of scanned roots divided by their dry mass, expressed in cm^2 g^{-1}) in each soil

sample. Fine-root mass density in each soil sample was calculated taking account of the soil dry mass used for the separation of the fine roots in the field and in the laboratory. Soil bulk densities measured in a pit down to a depth of 17 m in each treatment were used to convert fine-root mass densities expressed in g of root per kg of soil to g of root per dm^3 of soil in each layer (the same method was used from the soil surface down to a depth of 17 m). Fine-root biomasses were computed in each soil layer multiplying the soil layer volume (dm^3) by the mean fine-root density. Total root length and total root area in each soil sample were calculated by multiplying the total root dry mass by SRL and SRA, respectively. Root area index (RAI, surface area of fine roots divided by sampled soil area, expressed in $m^2\ m^{-2}$) and root length index (RLI, length of scanned roots divided by sampled soil area, expressed in km m^{-2}) (Jackson et al., 1997) were calculated for each soil layer in each treatment.

IX.2.4 Root ingrowth bags

Fine-root production over two periods of 3 months were studied using cylindrical ingrowth bags constructed from stainless steel with a mesh size of 2 mm, a diameter of 9 cm and a height of 35 cm. The 3-month periods of root regrowth were selected for very fast eucalypt fine root growth, with maximum ingrowth core colonization after 2 months, following a previous study at the same study site (see Figure.5 in Jourdan et al. (2008)). Three replicates of root ingrowth bags were installed at 4 depths (soil layers 0.10-0.45 m, 1.00-1.35 m, 3.00-3.35 m and 6.00-6.35 m) in the same plots used to study the fine-root densities (three treatments in three blocks) giving a total of 108 ingrowth bags for each period of 3 months. Holes (diameter 15 cm) were made manually (using the 'cavadeira' tool) to install the mesh bags at each depth (0.45 m, 1.35 m, 3.35 m or 6.35 m). The bags were filled with the soil from the corresponding soil layer and moistened to field capacity after removing all the roots. The flexibility of the bags allowed a good contact with the surrounding soil. PVC pipes (diameter of 0.15 m, length of 0.1 m, 1 m, 3 m or 6 m depending on the depth of the bag) were placed into the holes above the ingrowth bags to make possible to retrieve of each bag from the surface using a nylon cord attached (Appendix

Appendix IX. 1). A plastic bag (with a nylon cord attached) filled with about 2 kg of soil was placed between the root ingrowth bag and the bottom of the PVC tube to avoid air diffusion from inside the PVC tube to the ingrowth bags that could affect fine-root growth. The top of each PVC tube was hermetically sealed. The mesh size of 2 mm allowed fine-root growth. After a period of 3 months, the bags were carefully retrieved. We set up additional ingrowth cores at

the depths of 0.1 m and 1 m in the buffer rows of our experiment to check whether fine-roots were torn out when we pulled up the ingrowth cores 3 months after their installation. Destructive soil sampling close to the ingrowth cores showed that most of the fine roots of the two species were sheared by the stainless-steel mesh when the core was retrieved, and the biomass of fine roots not recovered inside the soil core was negligible. Two periods were studied: from July to September 2013 (winter) and October to December 2013 (spring). Just after the bags were retrieve, new bags were inserted at the same place to study the following 3-month period. All the fine roots in the samples were washed free of soil with tap water using sieves and separated carefully by hand. The roots of the two species were distinguished in the 50A50E treatment as described above.

IX.2.5 Statistical analyses

Two-way analyses of variance (ANOVAs) were used to assess the effects of treatments (100A, 100E and 50A50E) and blocks on fine-root densities (FRD), specific root length (SRL), specific root area (SRA), fine-root diameter, root length index (RLI) and root area index (RAI) for individual soil layers. Individual root biomass measurements within a given soil layer were considered independent since the sampling positions were located near different trees in each plot. We used linear mixed-effects models to test the effects of soil depth, treatment, and the interaction between depth and treatment (as fixed effect) on FRD, SRL, SRA, fine-root diameter, RLI and RAI for the whole soil profile. Blocks were considered as random effects and residues were modeled by a first-order autoregressive correlation model to account for the correlations between soil depths. A Shapiro-Wilk test was performed on the data before modeling and log-transformation was used when the residuals did not follow a normal distribution or were not homoscedasticity. In addition, two-way ANOVAs were carried out for each soil layer to compare the FRD of each tree species (*E. grandis* or *A. mangium*) in the mixed stands with 50% of the FRD in the monoculture of the same species in the same block. The effects of treatments and depth on FRD in the ingrowth bags were tested using two-way ANOVAs for each 3-month period studied. Post-hoc differences were analysed using Tukey's post-hoc Honest Significant Difference (HSD) to determine which means differed significantly between treatments. All calculations and analyses were performed using the R software version 3.2.2 (R development Core Team 2013) and the level of significance was 0.05. Linear mixed-effects models used the lmerTest package (Kuznetsova et al., 2015). For the graphical

representations, the mean values and standard errors were calculated from all the replicates (*n* = 9 down to a depth of 6 m and *n* =3 below).

IX.3 Results

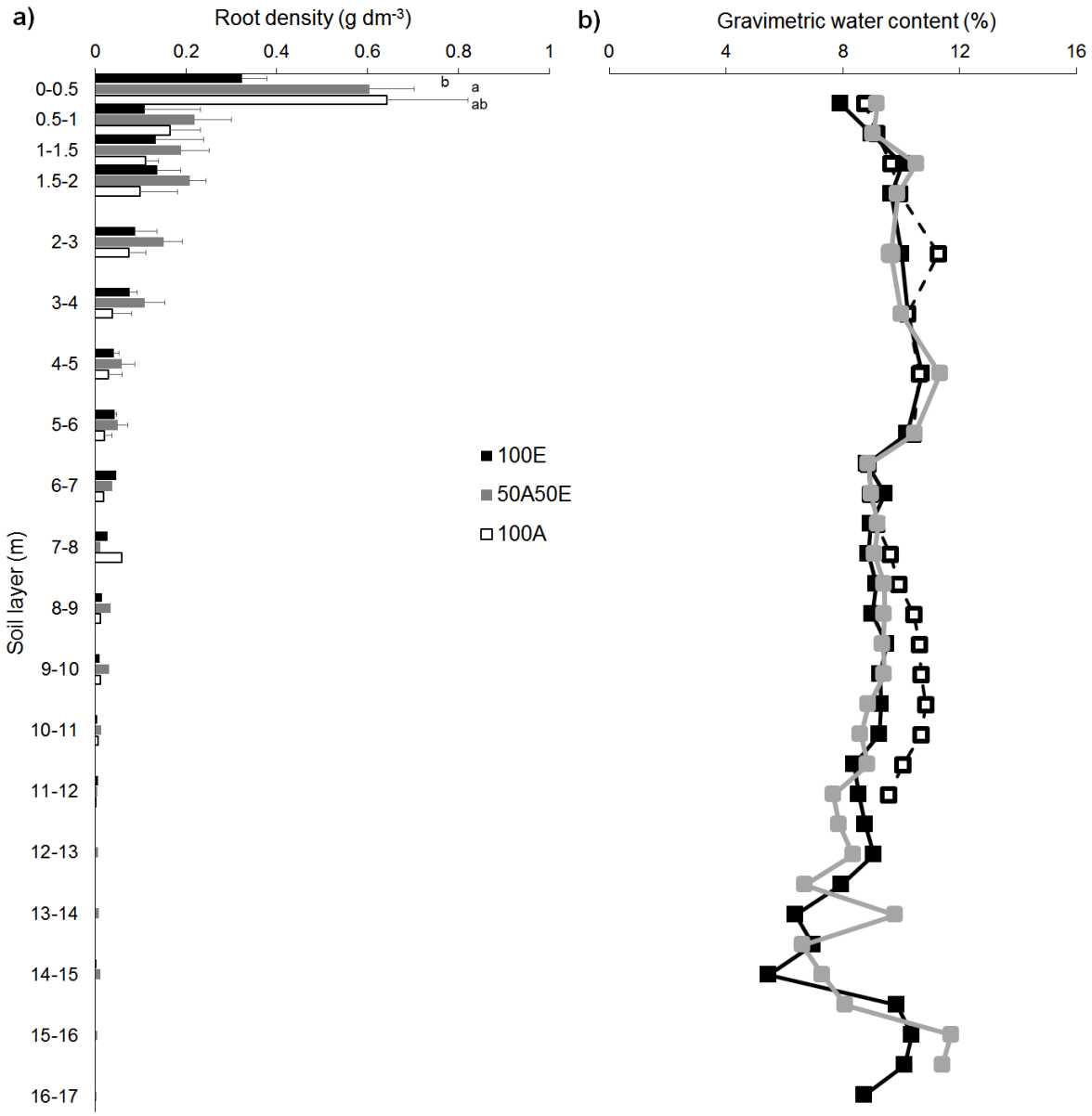
IX.3.1 Fine-root over-yielding

Auger sampling down to the root front (maximum depth of root observation) showed that trees in 50A50E, 100A and 100E exhibited a similar pattern of deep rooting. Fine-roots densities dropped sharply from the 0-0.5 m layer to the 0.5-1.0 m layer and decreased gradually down to a depth of 12 m in 100A and down to the water table at a depth of 17 m in 100E and 50A50E (Figure IX. 2). Mixing E. grandis and A. mangium led to fine-root over-yielding. Fine-root biomass in 50A50E (1127 g m^{-2}) was 44% higher than in 100A (780 g m^{-2}) and 58% higher than in 100E (714 g m^{-2}) (Table IX. 2). Fine-root densities were 20-100% higher in 50A50E than in 100E and 100A in most of the soil layers and were at least twice as high in 50A50E as in 100A in soil layers 1.5 - 2 m, 2 - 3 m, 3 - 4 m, and at depths > 8 m. Low fine-root densities at depths > 8 m in 100A were consistent with higher soil water contents in 100A than in 100E and 50A50E (Figure IX. 2).

Table IX. 2: Total fine-root biomass down to a depth of 17 m and mean specific root length (SRL), mean specific root area (SRA) and mean fine-root diameter down to a depth of 6 m in 100A, 100E and 50A50E at 4 years of age. Different uppercase letters indicate significant differences between treatments, and different lowercase letters indicate significant differences between the monospecific stands and the mixed stands for each species ($p < 0.05$).

	100A	**100E**	**50A50E**		
	A. mangium	*E. grandis*	*A. mangium*	*E. grandis*	Total
Fine-root biomass (g m^{-2})	779.68 B	714.19 B	421.94	696.93	1127.01 A
Specific root length (m g^{-1})	17.98 b	20.53 b	45.63 a	28.11 a	
Specific root area (cm^2 g^{-1})	268.36 b	253.57 b	584.11 a	338.55 a	
Diameter (mm)	0.53 b	0.47 a	0.48 a	0.47 a	

Figure IX. 2: Mean fine-root densities (a) and gravimetric water content (b) down to the root front in treatments 100E (*filled black bars*), 100A (*open bars*) and 50A50E (*filled grey bars*). Standard errors between blocks are given down to a depth of 6 m (n=3). Different letters indicate significant differences between treatments within each individual soil layer down to 6 m depth ($p < 0.05$, only significant in the upper soil layer).



IX.3.2 Fine-root distribution of each species in monospecific stands vs mixed stands

Intra- and inter-specific interactions strongly influenced the distribution of *A. mangium* and *E. grandis* fine roots, irrespective of the soil layer (Table IX. 3). The total fine-root biomass of *A. mangium* trees in 50A50E was 54% of the biomass in 100A, whereas the planting density was only 50% of that in 100A, with the slightly lower exploration of the 0-2 m soil layer than in 100A offset by a higher exploration of very deep soil layers (Figure IX. 3). *E. grandis* fine-root biomass was only 2% lower in 50A50E than in 100E, despite the stocking density being 50% lower (Figure IX. 3). *E. grandis* fine-root biomass in 50A50E was significantly higher than 50% of that in 100E in all layers except 1-2 m and 4–6 m. Even though the fine-root biomass was low between the depths of 9 and 12 m in all the treatments (about 6% of the total fine-root biomass), *A. mangium* and *E. grandis* fine-root biomasses at this depth were 2-3 times higher in 50A50E than in the monospecific stands where the stocking density of each species was twice as high (Figure IX. 3).

Figure IX. 3: Percentages of fine-root biomass in each soil layer in 50A50E relative to the fine-root biomass in the single-species stands. The *dotted line* indicates the 50% reference for *Acacia mangium* roots (open bars with standard errors) and *Eucalyptus grandis* roots (solid bars with standard errors), if root development was similar to the monoculture, for a 50% tree stocking density for each species. Standard error bars are shown. Asterisks * denotes a significant difference ($p<0.05$) between the root biomass of a particular species in 50A50E and 50 % of the biomass in the monoculture of the same species.

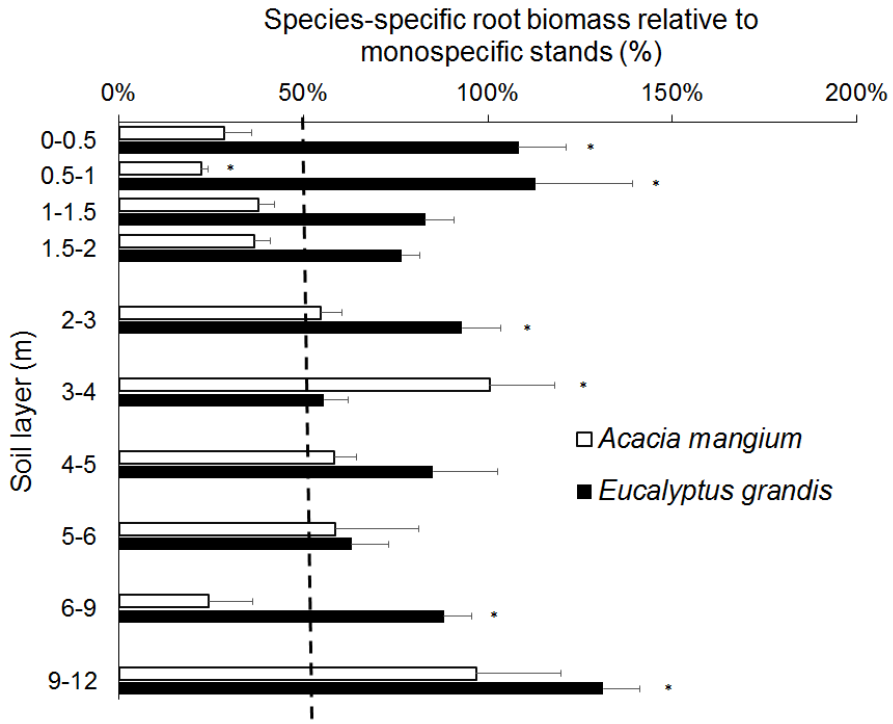


Table IX. 3: *P* values of linear mixed models for specific root length (SRL), specific root area (SRA) and root diameter applied to the whole soil profile between 100A and 50A50E for *A. mangium* roots and between 100E and 50A50E *E. grandis* roots as a function of treatment, soil depth and the interactions between factors.

	SRL		**SRA**		**Diameter**	
	A. mangium	*E. grandis*	*A. mangium*	*E. grandis*	*A. mangium*	*E. grandis*
Treatment	< 0.0001	0.0029	< 0.0001	0.0024	< 0.0001	0.889
Depth	0.3230	0.3238	0.675	0.6732	0.419	0.7353
Depth x Treatment	0.3854	0.5431	0.5988	0.8325	0.9029	0.9879

Table IX. 4: Fine-root biomass (g m^{-2}) in 100A, 100E and 50A50E. The percentage of root mass in each soil layer relative to the total root mass is in parentheses. Different letters indicate significant differences between treatments in each soil layer ($p < 0.05$).

Soil layer (m)	**100A**	**100E**	**50A50E**		
	A. mangium	*E. grandis*	*A. mangium*	*E. grandis*	Total
0-0.5	321 (41%) A	161 (23%) B	126 (30%)	173 (25%)	302 (27%) A
0.5-1	82 (11%) AB	54 (8%) B	33 (8%)	75 (11%)	109 (10%) A
1-1.5	55 (7%) B	67 (9%) B	33 (8%)	60 (9%)	94 (8%) A
1.5-2	49 (6%) B	68 (10%) B	35 (8%)	68 (10%)	104 (9%) A
2-3	74 (9%) B	89 (12%) B	58 (14%)	91 (13%)	151 (13%) A
3-4	38 (5%) C	76 (11%) B	45 (11%)	62 (9%)	108 (10%) A
4-5	29 (4%) A	41 (6%) A	23 (6%)	34 (5%)	58 (5%) A
5-6	20 (3%) B	42 (6%) A	16 (4%)	31 (4%)	48 (4%) A
6-9	90 (12%)	87 (12%)	31 (7%)	49 (7%)	81 (7%)
9-12	20 (3%) B	20 (3%) B	20 (5%)	25 (4%)	45 (4%) A
12-17	0	8 (1%)	1 (0.2%)	26 (4%)	27 (2%)
Total	780 (100%) B	714 (100%) B	422 (100%)	697 (100%)	1127 (100%) A

IX.3.3 Fine-root traits of each species in monospecific stands vs mixed stands

Total fine-root length index (RLI) was 30 km m^{-2} in 50A50E and was double that in 100A (13 km m^{-2}) and in 100E (15 km m^{-2}) (Figure IX. 5). The RLIs in soil layers 0-1 m, 2-4 m, 4-6 m and 9-17 m were significantly higher in 50A50E than in 100A and 100E. Total fine-root area index (RAI) was 28 m^2 m^{-2} in 50A50E and was significantly higher than in 100A (20 m^2 m^{-2}) and 100E (19 m^2 m^{-2}) (Figure IX. 5). While about 50% of the total RAI was found in the top 1 m soil layer in 50A50E (12.7 m^2 m^{-2}) and 100A (8.2 m^2 m^{-2}), only 22% of the total RAI was found in the top 1 m in 100E (4.2 m^2 m^{-2}) and a high proportion of the total RAI was in the 1-2 m soil layer (5.8 m^2 m^{-2}). The RAI at depths > 4 m was about 6 m^2 m^{-2}, irrespective of the treatment.

While specific root length (SRL) and specific root area (SRA) were significantly higher in 50A50E than in the monospecific stands, the mean fine-root diameter was significantly higher in 100A than in 100E and 50A50E (Table IX. 2, Table IX. 3). Down to a depth of 6 m, mean values of SRL were 18.0, 20.5 and 34.9 m g^{-1} in 100A, 100E and 50A50E, respectively, and the mean values of SRA were 268, 254 and 433 cm^2 g^{-1} (Table IX. 2). Mean fine-root diameter was 0.53 mm in 100A, and 0.47 mm in 100E and 50A50E. Depth had little effect on SRL, SRA and fine-root diameter for either species, irrespective of the treatment (Figure IX. 4).

Mixing *A. mangium* and *E. grandis* trees led to a significant increase in SRL and SRA for the roots of both species (Table IX. 2, Table IX. 3) while the mean fine-root diameter was significantly lower in mixed stands than in monoculture for *A. mangium* but not for *E. grandis*. SRL and SRA of *A. mangium* roots were 2 to 6 times higher in 50A50E than in 100A down to a depth of 2 m while the differences between treatments decreased at depths > 2 m (Figure IX. 4). The highest difference between the *A. mangium* root traits in 50A50E and in 100A was in the topsoil. In the top 0.5 m layer, the mean SRL of *A. mangium* fine roots was 72 m g^{-1} in 50A50E *vs* 11 m g^{-1} in 100A and the mean SRA was 848 cm^2 g^{-1} in 50A50E *vs* 187 cm^2 g^{-1} in 100A. Even though the mean diameter of *A. mangium* roots was higher in 100A than in 50A50E in all the soil layers down to a depth of 6 m, the differences between treatments were not significant in each individual layer (Figure IX. 4).

Figure IX. 4:Specific root length (a), specific root area (b) and mean fine-root diameter (c) in each soil layer for *Acacia mangium* (*left*) and *Eucalyptus grandis* (*right*) in monospecific stands and in 50A50E. Standard errors between blocks are indicated (n=3). Asterisks * denotes a significant difference ($p<0.05$) between 50A:50E and the monoculture.

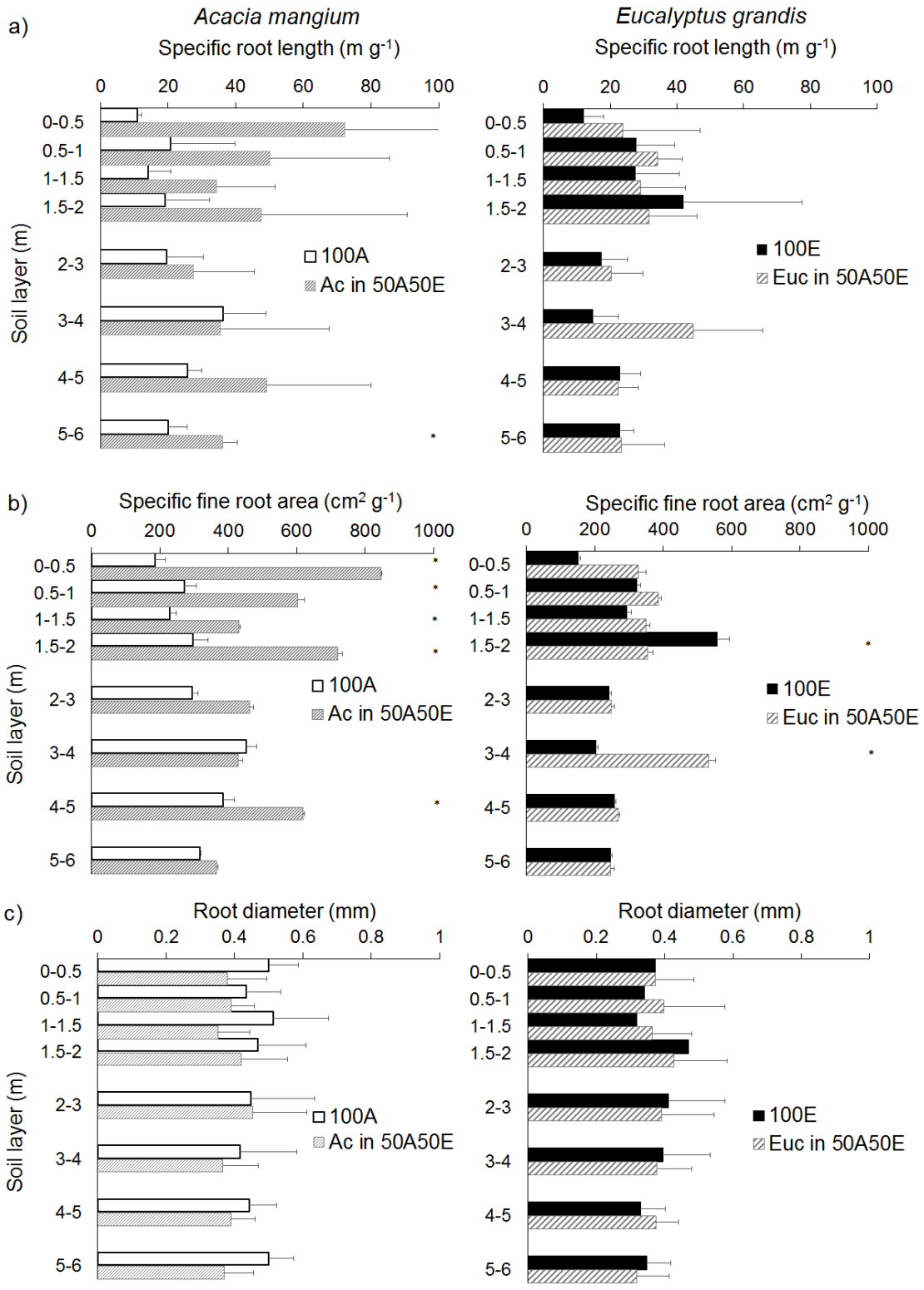
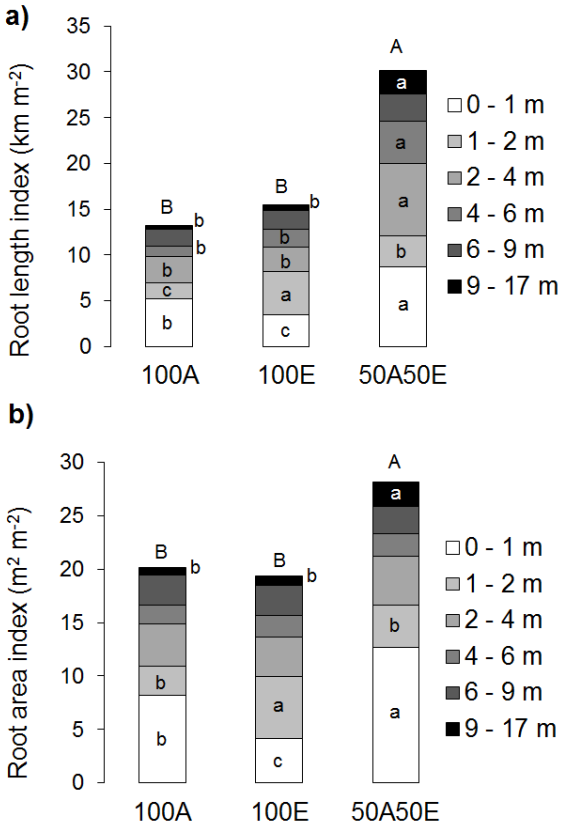


Figure IX. 5:Root length index (km m^{-2}) a) and root area index (m^2 m^{-2}) b) in the 0-1 m, 1-2 m, 2-4 m, 4-6 m, 6-9 m and 9-17 m soil layers for *Acacia mangium* monospecific stands (100A), *Eucalyptus grandis* monospecific stands (100E) and the mixed stands (50A50E). Different upper-case letters indicate significant differences between treatments for the cumulative indices and different lower-case letters indicate significant differences between treatments within each individual soil layer ($p < 0.05$).

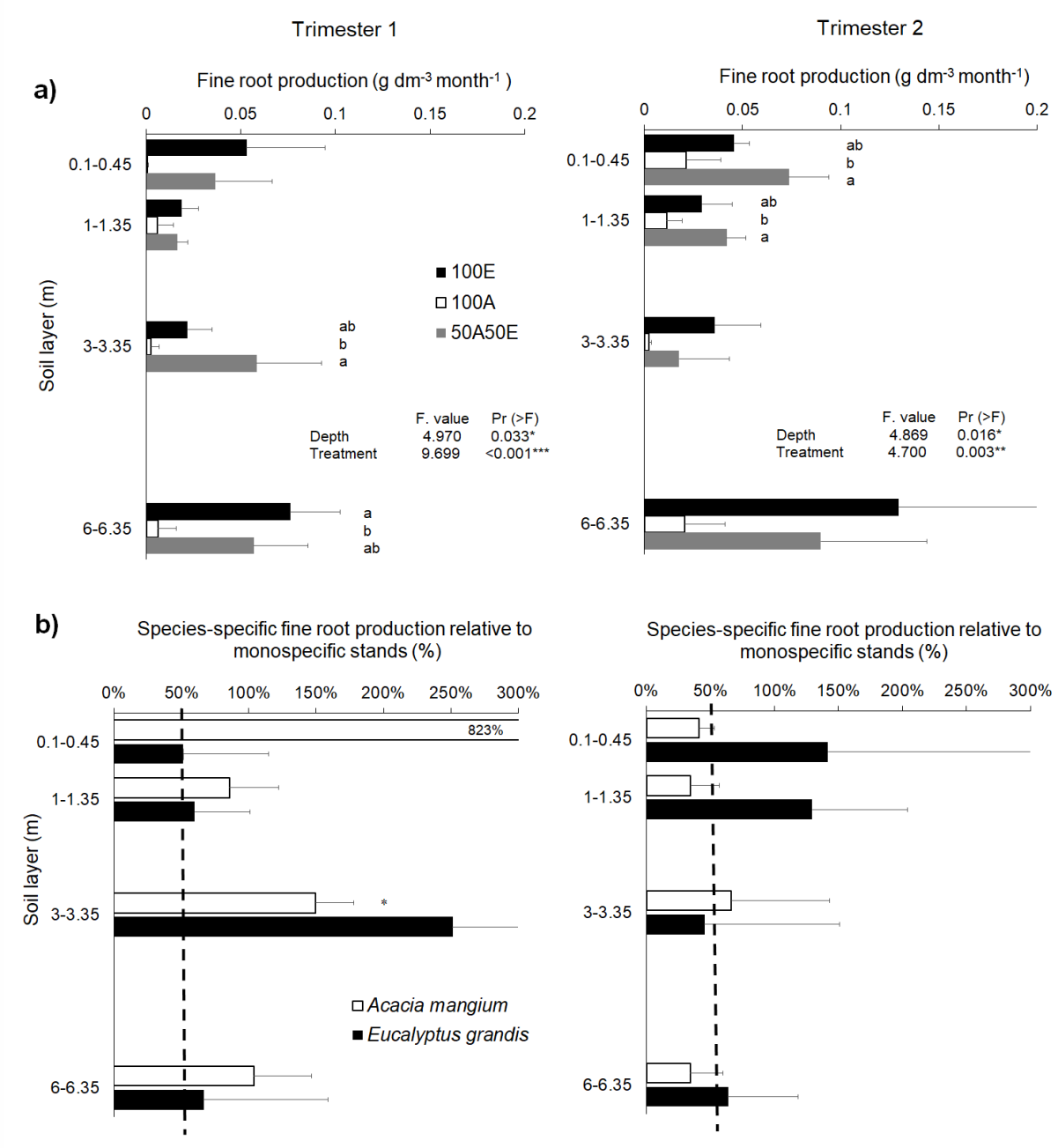


IX.3.4 Capacity to explore soil patches

Mean fine-root production in the ingrowth bags reached 0.048 ± 0.025 g dm^{-3} $month^{-1}$ in 50A50E across the two trimesters and the four depths studied and was 63% higher than the average of 100A and 100E (Figure IX. 6). Surprisingly, the effect of soil depth on fine-root production in the ingrowth bags was not significant. Fine-root production in the ingrowth bags increased by 268% in 100A, 42% in 100E and 33% in 50A50E from the first 3-month period (winter, dry season) to the second (spring). Fine-root production was significantly higher in 100E and 50A50E than in 100A. In individual soil layers, fine-root production was not significantly different between 50A50E and 100E, but significantly higher than in 100A at depths 3-3.35 m and 6-6.35 m in winter and at depths 0.1-0.45 m and 1-1.35 m in spring.

The fine-root production of each species in 50A50E and 50% of the production in monospecific stands showed a strong seasonality (Figure IX. 6). While the production of *A. mangium* roots in winter in the ingrowth bags tended to be higher in 50A50E than 50% of that produced in 100A, it was lower in spring. The production of *E. grandis* roots in the ingrowth bags in winter was slightly higher in 50A50E than 50% of the root biomass produced in 100E in soil layers 1 - 1.35 m and 6 - 6.35 m, and 5 times higher in the 3 - 3.35 m soil layer. In spring, the production of *E. grandis* roots in the ingrowth bags was 2-3 times higher in 50A50E than 50% of the production in 100E in the upper soil layers (0.1 - 0.45 m and 1 - 1.35 m depths) and close to 50% of the root production in 100E in deep soil layers (3 - 3.35 m and 6-6.35 m depths).

Figure IX. 6: Fine root production (g dm^{-3} $month^{-1}$) in ingrowth bags down to a depth of 6.35 m in 100E (*filled black bars*), 100A (*open bars*) and 50A50E (*filled grey bars*) (a) and percentages of fine-root production in 50A50E relative to the production in each soil layer for the single-species stands (b) in winter (dry season; Trimester 1, *left*) and in spring (Trimester 2, *right*). Significant differences between treatments in the same soil layer are indicated by different letters ($p<0.05$). The *dotted lines* indicate the 50% reference for *Acacia mangium* roots (open bars with standard errors) and *Eucalyptus grandis* roots (solid bars with standard errors) if root development was similar to the monoculture, for a 50% stocking density for each species. Standard error bars are shown (n=3). Asterisks * denotes a significant difference ($P<0.05$) between the root biomass of a particular species in 50A50E and 50 % of the biomass at the same positions in the pure stands of the same species. F and *P* values of linear mixed models for fine-root production (g dm^{-3} $month^{-1}$) as a function of soil depth and treatment are shown.



IX.4 Discussion

IX.4.1 Fine-root over-yielding in very deep soil layers

The main purpose of this study was to investigate whether roots explore very deep soil layers more intensively in mixed-species stands than in monospecific stands. In agreement with our first hypothesis, mixing *A. mangium* and *E. grandis* trees led to a strong fine-root over-yielding in all soil layers down to the water table at a depth of 17 m. Although fine-root over-yielding has already been reported in the upper soil layers for mixed stands compared to monospecific stands (Brassard et al., 2013; Laclau et al., 2013c; Lei et al., 2012), the opposite has also been reported (Bolte & Villanueva, 2006), and other studies have shown similar fine-root biomasses in mixed and monospecific stands (Bauhus et al., 2000; Meinen et al., 2009). A recent global meta-analysis across forests, grasslands, croplands and pot systems showed that mixed stands had, on average 28% higher fine-root biomass and 45% higher annual production than monocultures (Ma & Chen, 2016). The originality of our results come from the strong fine-root over-yielding observed in soil layers at depths > 2 m. Competition for water and nutrients with the neighboring trees in mixed stands may force the roots to explore and tap deep soil layers (Cardinael et al., 2015; Ma & Chen, 2017). While most of the studies dealing with fine-root distribution in forests have been limited to the upper 2 m of soil, 35%, 45% and 50% of the total fine-root biomass was found below 2 m in 100A, 50A50E and 100E, respectively. The difficulty of sampling deep roots can lead to underestimates of root mass and belowground productivity in tropical plantations and forests (Maeght et al., 2015b; Pierret et al., 2016b). Studies quantifying total fine-root biomass down to the root front are scarce for trees growing in very deep tropical soils. Close to our study site (on the same soil type), the proportion of fine roots below 2 m depth was 20-30% of the total fine-root biomass down to the root front in a sweet orange orchard (Adriano et al., 2017) and 30-60% of the total fine-root length, depending on tree age, in eucalypt plantations (Laclau et al., 2013b; Pinheiro et al., 2016a). As commonly reported in forest ecosystems, fine-root densities dropped rapidly within the first topsoil layers then decreased gradually with soil depth, which corresponds to the distribution of nutrients in soil profiles (Weemstra et al., 2017). Interactions between tree species greatly increase the exploration of very deep soil layers in our study, which is likely to enhance the uptake of soil resources. Establishing a deep root system can also help tropical trees withstand the prolonged droughts that are expected to increase in the future (Christina et al., 2017a; Solomon et al., 2009). A modeling approach in a nearby eucalypt stand of similar productivity showed that

very low densities of fine roots at depths > 10 m can withdraw substantial amounts of water during dry periods (Christina et al., 2017a). Mixing tree species could therefore enhance the resilience of tropical planted forests to climate changes increasing the access to water and nutrients stored in very deep soil layers.

Fine-root over-yielding in 50A50E is also consistent with the higher soil respiration rates relative to 100A and 100E at the end of the previous rotation (Nouvellon et al., 2012b). Roots are a major contributor to soil respiration (Gill et al., 2002; Marsden et al., 2008) and about 25% of terrestrial net primary production is due to fine-root production and turnover (McCormack et al., 2015). The total belowground C fluxes from 4 to 6 years after planting in the previous rotation of our experiment represented 24%, 26% and 32% of the gross primary production in 100A, 100E and 50A50E, respectively (Nouvellon et al., 2012b). Large amounts of C are stored at great depths in tropical soils (Batjes, 2014) and changes in land use increasing the supply of fresh carbon might promote the activity of microbes through a priming effect (Derrien et al., 2014b; Fontaine et al., 2007). The increase in fine-root biomass, by replacing monospecific stands by mixed-species, may have consequences on soil carbon sequestration in deep soil layers that are poorly understood. Further studies are needed to assess whether an increase in the release of fresh C in very deep soil layers resulting from the combination of tree species could contribute to mitigating climate change through a sequestration of C or, on the contrary, would promote the mineralization of ancient C and therefore increase CO_2 emissions.

IX.4.2 Root strategies in response to intra- and inter-specific interactions

In agreement with our second hypothesis, the interaction mechanisms between *E. grandis* and *A. mangium* trees led to large changes in *A. mangium* fine-root morphology. Previous studies at the same experimental site showed that *E. grandis* trees dominate the competition with *A. mangium* trees capturing more light per tree and taking advantage of N fixation (Paula et al., 2015). The *A. mangium* leaves are below the canopy of eucalypt trees in mixed stands and fine roots are partially excluded from the upper soil layer (Laclau et al., 2013c; Le Maire et al., 2013). *A. mangium* trees adopt an intensification strategy (Bonifas & Lindquist, 2009; Lei et al., 2012; Ostonen et al., 2007a) in competition with eucalypts, making it possible to increase the capacity to take up soil resources for a relatively low investment in belowground biomass. SRL and SRA of *A. mangium* fine roots in mixed stands were 2-3 times higher than in monoculture, which, in combination with a large increase in FRD, led to a much higher root length index and root area index. While some studies in forest ecosystems also showed higher

SRL and SRA in mixed stands than in monospecific stands, making it possible to reduce the cost/benefit ratio for resource capture (Beyer et al., 2013; Lei et al., 2012; Sun et al., 2017; Weemstra et al., 2016), those root traits were similar in mixed-species plantations and monocultures of *A. mearnsii* and *E. globulus* in Australia (Bauhus et al., 2000). In our study, *A. mangium* faced competition with *E. grandis* by altering the root absorption capacity, much more through morphological adaptations than changing the fine-root biomass of each *A. mangium* tree.

C starvation affecting the fine root architecture of *A. mangium* trees dominated by eucalypts as well as changes in mycorrhizal status seem to be potential factors. A recent study carried out in the same experiment showed large changes in the microbiological and chemical attributes of soil between the 100A and 50A50E treatments (Bini et al., 2013), which suggests that changes in mycorrhizal status might be contribute to explaining the effect of eucalypts on the SRL and SRA of *A. mangium* roots. However, the mycorrhizal status was not directly measured, and we can only speculate about this effect. Differences in nodulation might contribute to explaining this pattern. Indeed, nodule density was much higher in monospecific *A. mangium* stands than in mixed *A. mangium* and *E. grandis* stands in the previous rotation at this site (Bouillet et al., 2008). However, nodules were mainly observed in the 0-50 cm soil layer and their density was low in the scanned roots.

Our results confirm the fine-root segregation between the two species in the mixed stand observed for the previous rotation at this site (da Silva et al., 2009; Laclau et al., 2013c), with a partial exclusion of *A. mangium* roots from the resource-rich upper soil layers in mixed stands and a higher exploration of deep soil layers. However, the methodology was less intensive than in the previous studies of this effect (only one distance to the trees was sampled here and the 0-50 cm soil layer was not divided in several layers).

Eucalyptus grandis trees respond to competition with *A. mangium* trees through both an extensification strategy, leading to a sharp increase in fine-root biomass relative to monospecific stands, and an intensification strategy, increasing the volume of capture of soil resources relative to the fine-root biomass. However, the changes in SRL, SRA and fine-root diameter were non-significant in most of the soil layers, which suggests that the contribution of the intensification strategy was low. SRL and SRA values in our study are in the range reported down to a depth of 12 m for four *Eucalyptus* genotypes in nearby plantations (Pinheiro et al., 2016a). The total biomass of *E. grandis* fine roots was similar in 50A50E and in 100E despite

a stocking density of eucalypt trees twice as high in 100E, which shows a remarkable plasticity of eucalypt trees to explore a huge soil volume, as already shown in Laclau et al. (2013c). In boreal forests, a comparison of fine-root production in mixed and single-species stands showed that fine-root production was correlated with nitrogen concentrations in the soil layers, and suggested that an enhancement of N availability in the mixed stands contributed to increasing fine-root production (Ma & Chen, 2017). We, therefore, speculate that facilitation mechanisms for *E. grandis* trees planted with *A. mangium* resulting from an increase in soil N availability (Tchichelle et al., 2017; Voigtlaender et al., 2012), could be involved in the extensification strategy leading to an increase in soil exploration by *E. grandis* fine roots. Such pattern might be pronounced in the superficial soil layers where eucalypt trees benefit from a rapid belowground N transfer from *A. mangium* trees (Paula et al., 2015).

While many studies show that SRL, SRA and fine-root diameter can change depending on soil depth (Bakker et al., 2009; Makita et al., 2011; Maurice et al., 2010; Prieto et al., 2015), we did not observe clear patterns down the soil profile for either species. The same type of study carried out down to the root front in other eucalypt and orange plantations in Brazil also showed a high variability of SRL, SRA and fine-root diameter between soil layers but without a clear correlation with the depth (Adriano et al., 2017; Pinheiro et al., 2016a). A study carried out in 20 plant communities sampled in tropical, Mediterranean and montane regions showed that some fine-root traits were significantly different between shallow and deep soil layers, but absolute differences were small for most of the traits measured (Prieto et al., 2015). Our results suggest that *E. grandis* and *A. mangium* fine roots in mixed stands reflect an acquisitive resource strategy (Freschet et al., 2017; McCormack et al., 2015). Further studies in eucalypt and acacia plantations should examine other important root traits (in particular tissue density, concentrations of nutrients, cellulose, lignin and carbohydrate) to gain insight into the cost to the trees of investing in fine-root production in very deep soil layers.

IX.4.3 Fine-root production

Even though the fine-root production of *A. mangium* and *E. grandis* trees exhibited a strong seasonal variability, the decrease in fine-root density with soil depth did not modify the production of fine roots in the ingrowth bags down to a depth of 6 m. This pattern is contrary to our third hypothesis and shows a huge capacity of very deep roots to explore resource-rich soil patches despite their low density in the soil. The production of fine roots in the ingrowth bags was however much higher for *E. grandis* trees than for *A. mangium* trees, which is

consistent with the faster growth rates of *E. grandis* trees shown both aboveground and belowground (da Silva et al., 2009; Nouvellon et al., 2012b). Fine-root production in the ingrowth bags confirmed the strong over-yielding in mixed-species stands relative to the monocultures suggested by the fine-root densities. However, fine roots sampled in ingrowth bags can be different from fine roots sampled in undisturbed soil and productions estimated from ingrowth bags should be interpreted with caution (Bauhus & Messier, 1999; Jourdan et al., 2008). Nevertheless, the same ingrowth bags were installed at all the depths in all the treatments, making it possible to compare the capacity of fine roots to explore soil patches.

The production of *A. mangium* fine roots in the ingrowth bags was much more affected by the season (winter *vs* spring) in the topsoil than in deep soil layers, as reported for phreatophytic species (Canham et al., 2012). *E. grandis* trees are better adapted to the cold climate in winter at our study site than *A. mangium* trees, which could account for the higher fine-root production in the topsoil in *E. grandis* monoculture and mixed-species stands than in *A. mangium* monoculture. A strong influence of exogenous factors such as soil temperature and water content (Canham et al., 2015), as well as endogenous factors such as photosynthate availability, on fine-root phenology is well documented (McCormack et al., 2015).

In conclusion, our study shows that mixing species can lead to a strong fine-root over-yielding in very deep soil layers. We demonstrate that *E. grandis* and *A. mangium* trees growing in mixed stands can explore more intensively deep soil layers than their respective monospecific stands. Studies dealing with deep rooting are needed for other tree species, soils and climates to assess whether increasing the diversity in tropical planted forests could increase their resilience to climate change by providing access to more soil resources than monospecific stands. A better comprehension of the effects of fine-root growth and turnover in very deep soil layers on soil carbon stocks is also needed to better assess the long-term consequences of afforestation with fast-growing tree species on the global carbon cycle.

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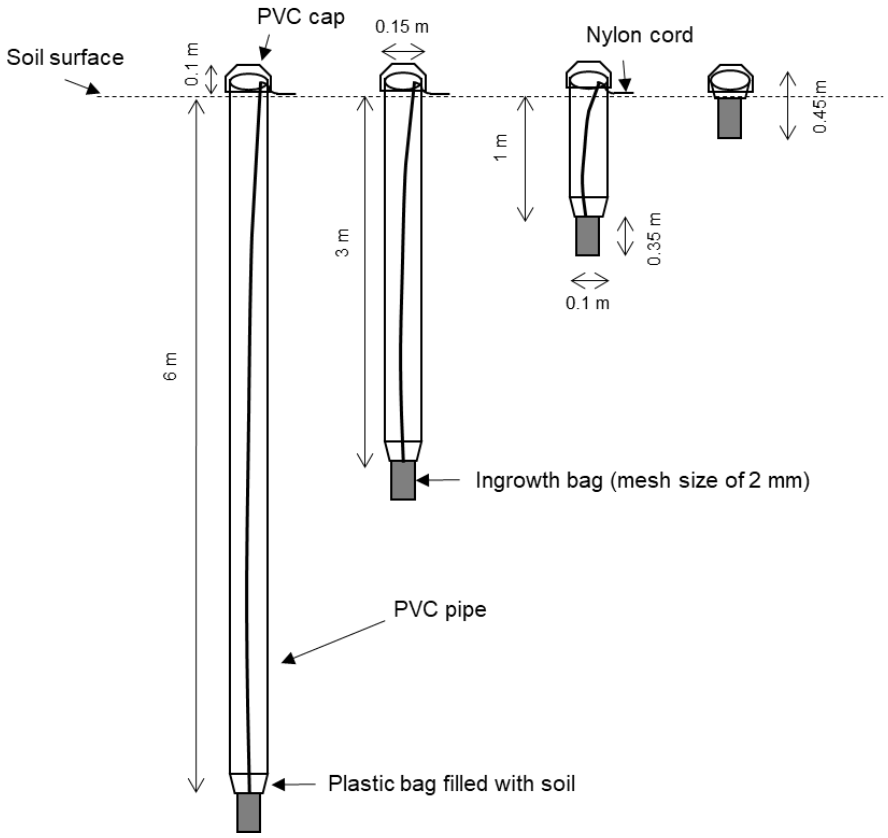
Appendix

Appendix IX. 1: Root ingrowth bags at the four sampled depths



Consequences of clear-cutting on the production of fine roots, CO_2, CH_4 and N_2O down to the water table in *Eucalyptus grandis* stands conducted in coppice in a throughfall-exclusion experiment.

Improving our understanding of the spatiotemporal dynamics of fine roots and greenhouse gases in deep soil layers is a key component to identify more sustainable silvicultural practices for planted forests in a context of climate change and to improve the current biogeochemical models. Our study aimed to assess the effect of clear-cutting and drought on fine-root production, soil CO_2, CH_4 and N_2O effluxes and production throughout deep soil profiles down to the water table in Brazilian coppice-managed *Eucalyptus* plantations. Fine roots (i.e. diameter < 2 mm) were sampled down to a depth of 17 m in a throughfall exclusion experiment comparing stands with 37% of throughfall excluded by plastic sheets (-W) and stands without rain exclusion (+W). Root dynamics were studied using minirhizotron in two permanent pits down to a depth of 17 m in treatments -W and +W, over 1 year before clear-cutting, then over 2 years in coppice, as well as down to a depth of 4 m in a non-harvested plot (NH) serving as a control. CO_2, CH_4 and N_2O surface effluxes were measured over three years using the closed-chamber method in treatments -W, +W and NH. CO_2, CH_4 and N_2O concentrations in the soil were measured from the pits down to a depth of 15.5 m in treatments -W, +W and NH over 3 months before the clear-cut and 1.5 years after in coppice. After harvesting, spectacular fine root growth of trees conducted in coppice occurred in very deep soil layers (> 13 m) and, surprisingly, root mortality remained extremely low whatever the depth and the treatment. Total fine-root biomass in coppice down to a depth of 17 m was 1266 and 1017 g m^{-2} in treatments +W and -W, respectively, at 1.5 years after the clear-cut and was 1078 g m^{-2} in NH 7.5 years after planting. Specific root length and specific root area were about 15% higher in -W than in +W. CO_2, CH_4, and N_2O effluxes were not significantly different between treatments -W and +W and did not change after clear-cutting in the coppice-managed stands compared to non-harvested stand. CO_2 and CH_4 concentrations greatly increased with depth and N_2O concentrations remained roughly constant from the soil surface down to a depth of 15.5 m. Mean CO_2 and N_2O concentrations in -W were 20.7% and 7.6% lower than in +W, respectively, and CH_4 concentrations in -W were 44.4% higher than in +W throughout the soil profiles. A diffusivity model showed that CO_2, N_2O and CH_4 production and consumption occurred at great depths and were similar in treatments +W, -W and NH. Clear-cutting did not increase CO_2, CH_4 and N_2O effluxes and productions, whatever the water supply regime. Establishing deep root systems in tropical planted forests could help trees withstand the long drought periods expected under climate change in many tropical regions. Our study suggested that coppice management might be an interesting option in tropical *Eucalyptus* plantations, both to improve tree tolerance to drought and store carbon at great depth in the soil.
Keywords: Brazil, *Eucalyptus grandis*, deep root growth, greenhouse gases, throughfall exclusion, very deep tropical soil, coppice

Conséquence de la coupe rase sur la production de racines fines, CO_2, CH_4 et N_2O jusqu'à la nappe phréatique dans une plantation d'*Eucalyptus grandis* menée en taillis sur un dispositif d'exclusion de pluie.

Améliorer notre compréhension de la dynamique spatio-temporelle des racines fines et des gaz à effet de serre dans les couches profondes du sol est un élément clé pour identifier des pratiques sylvicoles plus durables pour les forêts plantées dans un contexte de changement climatique et pour améliorer les modèles biogéochimiques actuels. Notre étude visait à évaluer l'effet de la coupe des arbres et de la sécheresse sur la production de racines fines et les émissions et production de CO_2, N_2O et CH_4 sur un profil de sol profond jusqu'à la nappe phréatique, dans des plantations d'*Eucalyptus* menées en taillis au Brésil. Les racines fines, d'un diamètre inférieur à 2 millimètres, ont été échantillonnées jusqu'à une profondeur de 17 m sur un dispositif d'exclusion de pluie comparant des peuplements soumis à une exclusion de 37% des pluviolessivats (-W) et des peuplements sans exclusion (+ W). La dynamique des racines a été étudiée à l'aide de minirhizotrons installés dans deux fosses permanentes d'une profondeur de 17 m dans les traitement -W et + W, pendant un an avant la coupe des arbres, puis pendant deux ans en taillis, et jusqu'à 4 m de profondeur dans un peuplement non récolté (NH) servant de témoin. Les flux de CO_2, de CH_4 et de N_2O à la surface du sol ont été mesurés durant trois ans en utilisant la méthode des chambres manuelles dans les traitements -W, + W et NH. Les concentrations de CO_2, de CH_4 et de N_2O dans le sol ont été mesurées à partir des fosses jusqu'à une profondeur de 15.5 m dans les traitements -W, + W et NH durant 3 mois avant la coupe des arbres et 1.5 ans après, en taillis.
Après la coupe, nous avons observé une croissance spectaculaire des racines fines en grande profondeur (> 13 m) chez les arbres menés en taillis et, étonnamment, une mortalité extrêmement faible des racines quelle que soit la profondeur et le traitement. La biomasse totale des racines fines jusqu'à 17 m de profondeur dans les traitements menés en taillis était de 1266 et 1017 g m^{-2} dans + W et -W, respectivement, 1.5 an après la coupe des arbres et de 1078 g m^{-2} dans le traitement NH, 7.5 ans après la plantation. La longueur spécifique et la surface spécifique des racines étaient environ 15% plus élevées dans -W que dans + W. Les flux de CO_2, de CH_4 et de N_2O ne différaient pas significativement entre -W et + W et ne changeaient pas après la coupe des arbres dans les peuplements menés en taillis par rapport aux peuplements non exploités. Les concentrations de CO_2 et de CH_4 augmentaient fortement avec la profondeur et les concentrations de N_2O restaient à peu près constantes de la surface du sol jusqu'à une profondeur de 15.5 m. Les concentrations moyennes de CO_2 et de N_2O dans -W étaient respectivement inférieures de 20.7% et 7.6% à celles de + W et celles de CH_4 dans -W de 44.4% supérieures à celles de + W sur tout le profil de sol. Un modèle de diffusivité a montré que la production et la consommation de CO_2, de N_2O et de CH_4 se produisaient à de grandes profondeurs et étaient similaires dans les traitements + W, -W et NH. La coupe des arbres n'a pas augmenté les flux et les productions de CO_2, de CH_4 et de N_2O, quel que soit le régime hydrique. Notre étude suggère que la gestion en taillis permet de conserver et de réexploiter le système racinaire mis en place par les Eucalyptus lors de la rotation précédente. Cette gestion est une solution pour l'atténuation des changements climatiques car elle pourrait aussi être une option intéressante d'adaptation des plantations tropicales aux changements climatiques car elle permet d'améliorer la tolérance des arbres à la sécheresse et de stocker du carbone en plus grande profondeur dans le sol.
Mots clés : Brésil, *Eucalyptus grandis*, croissance racinaire en profondeur, gaz à effet de serre, exclusion de pluie, sol tropical très profond, taillis